STUDIES ON THE ECOLOGY AND TAXONOMY OF THERMOPHILOUS FUNGI

by

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Abstract

An ecological study of thermophilous fungi was undertaken, with particular reference to their occurrence in the coal spoil tips in the Stoke-on-Trent area.

A wide variety of fungal species was isolated during these investigations, and species were divided into groups depending on their frequency of isolation (and occurrence) in all the habitats sampled. The warm areas of coal spoil tips were shown to be particularly rich in their thermophilous mycofloras and it is considered that these habitats may favour the growth of thermophilous fungi and encourage colonisation by fungi poorly adapted to normal soil conditions.

The seasonal variation of thermophilous fungi in a coal spoil tip profile and in the atmosphere was also investigated. In the former habitat the numbers of fungal isolates and species were found to decrease markedly with depth, and in both habitats there was a definite seasonal fluctuation in these numbers.

The temperature-growth relationships of the isolated species were determined in detail and, on the basis of these, species have been delimited into groups. Several

species have been added to the small number of true thermophiles so far reported. Problems concerning the definition of thermophily are discussed.

A taxonomic survey of thermophilous fungi has been made and several species new to the British Isles are described. One new species is named and a number of unusual species are described and names proposed for several of them. Several new combinations are also thought to be necessary.

The cellulolytic ability of a range of thermophilous fungal species was measured using two different methods. Species are divided into groups depending on their degree of cellulolytic ability.

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I N T R O D U C T I O N

Cooney and Emerson (1964) described all the then previously known thermophilic fungi in great detail and in consequence their monograph helped to stimulate the present study. Thirteen species were listed and the authors concluded that further studies embracing wider habitats would undoubtedly bring to light new thermophilic species of fungi. Indeed, in their addendum they list several new species and different sources of known thermophiles reported just prior to the publication of their monograph. This demonstrates the increasing interest being shown in these unusual fungi.

A historical survey of the thermophilic fungi has been well documented (Crisan 1959, Cooney and Emerson 1964) and, therefore, only the more recent or relevant studies will be discussed. Crisan (1959) and several previous workers concluded that thermophilic fungi are poorly represented in the soil. However, Apinis (1960, 1963a, 1963b) isolated a variety of known thermophiles and several new species from a few selected natural habitats. The latter author (1960) discussing the distribution of thermophilic fungi stated that "their presence in the surface layers of the soil and in plant debris appears to be a natural phenomenon because these

are periodically warmed up by the radiation of the sun and thus suitable conditions are created for their development There is good evidence to support the effect of solar insolation in dramatically increasing the temperature of surface soils. Vaartaja (1954) measured the summer temperature of forest sites in Finland. He found that little differences existed in air temperature at a height of 1.8 metres above the ground, whereas the temperature conditions on the same sites differed greatly in the thin surface soil. The sites under dense spruce canopies scarcely warmed by day and then mainly by convection, whilst the less dense pine sites warmed considerably due to insolation. Maximum surface temperatures of 50-70°C were recorded and considered to be of normal occurrence in forest soil.

e.g.	max. °C.	average noon
a. 1-3 cm. thick humus and litter	64	62
b. 2 mm. thick forest humus	59	52
c. White sand	42	38

These results are from measurements on exposed areas in the thin surface soil during July when the air temperature at 1.8 metres was 20°C.

Ranzoni (1968) measured air and soil temperatures

in the Sonoran desert, North America. The minimum July air temperature for a five day period was 23°C and the maximum was 40.6°C. The minimum soil temperature for the same period was 27°C whilst the maximum was 58°C. The consistently higher figures for soil temperature in relation to air temperature demonstrates clearly the effect of solar insolation. However, the latter author failed to isolate any fungi on plates incubated over 45°C, a temperature well within the soil temperature range. Nevertheless, the occurrence of high soil temperatures due to insolation provides an equable habitat for thermotolerant and thermophilic fungi. The latter, perhaps, have an ephemeral life cycle, especially in temperate climates.

Craveri et al. (1964) investigated the thermophilic and thermotolerant fungi of common soils and came to the conclusion that their presence in the soil does not appear to be particularly associated with thermal conditions. However, they considered that in natural environments high temperatures may occur and thus enhance the growth of these micro-organisms. They isolated fifty strains of eumycetes from cultivated soils collected from different regions of Northern Italy. Only twenty-four were considered to be true thermophiles, the remainder

being thermotolerants. They listed the thermophilic genera; Thermomyces, Talaromyces, Thermoascus, Chaetomium and Thermoidium whilst the thermotolerants belonged mainly to the genera Mucor and Aspergillus. The minimum temperature which they selected for the growth of a thermophile was slightly higher than that defined by Cooney and Emerson (20°C) being 25°C or above. The standards which the present writer adopts and the reasons for their selection will be discussed later in the chapter on temperature relationships (Chapter IV). Craveri et al. (1967) continued the study of thermophiles of normal soils and isolated twelve 'strains' of eumycetes able to grow at unusually high temperatures (50-60°C), and on the basis of their previous definition of thermophily divided them into thermotolerants (thermofacultatives) and thermophiles. Mucor pusillus Lindt and Humicola insolens Cooney and Emerson which were originally classed as true thermophiles are described by Craveri et al. as being thermofacultative, on the basis of their ability to grow below 25°C. Hence, there is still confusion regarding the working definition of thermophily despite the great care taken by Cooney and Emerson to remedy this confusion.

Previous workers have tested the effect of elevated

temperatures on the isolation of soil fungi. Timonim (1935), Bisby, Timonim and James (1935) incubated dilution plates at 37°C and demonstrated that temperature had a selective effect on the number and types of fungi isolated. The latter authors showed that there were exceptionally large numbers of Aspergilli but relatively few Penicillia. Also certain fungi, such as Humarina, Xylaria and Haplographium, not usually found in soil cultures appeared. Most of the high-temperature fungi were found at or near the surface of the soil which would be subjected to the highest summer temperatures. Greaves and Jones (1944) also showed the influence of temperature on the microflora of the soil without, however, making a qualitative analysis of the isolated micro-organisms. They stored soils at different temperatures for twenty-four months and found that the greatest number of micro-organisms developed when the soils had been stored at 10°C, and the fewest at 40°C. However, it is not until more recent times that higher isolation temperatures have been adopted and the even greater influence that temperature exerts on the numbers and types of fungi isolated from soil plates demonstrated.

Küster and Locci (1964) studied the occurrence of thermophilic fungi in peat by using isolation temperatures

of 45°C and 55°C. The numbers of species isolated was very small compared with those isolated at lower temperatures. Mucor pusillus, Aspergillus fumigatus, Humicola insolens, Humicola stellata, Thermomyces lanuginosus and a Paecilomyces species were shown to be present. The occurrence of these thermotolerant and thermophilic fungi can be correlated with the high temperatures often encountered in peat piles. More difficult to explain is the presence of thermotolerant and thermophilic fungi in habitats less likely to show abnormal temperatures. Apinis (1963a) and Apinis and Chesters (1964) isolated from coastal grasslands several ascomycetes which showed varying degrees of thermophily. These included Allescheria terrestris, Anthostomella sp. A, Byssochlamys nivea, Chaetomium funicola, C. thermophile, Dactylomyces crustaceus, Emericella nidulans, Sartorya fumigata, Talaromyces duponti and Thielavia sepedonium. All were originally isolated at 38°C but certain of these species would be eliminated at higher incubation temperatures.

There are, therefore, relatively few workers who have analysed the distribution of thermophilic fungi in normal soils, and most attention has been focused on habitats favouring the growth of thermophiles but which

can be described as semi-natural, e.g. manures and composts. Fergus (1964) reported many of the known thermophilic fungi and one new species, Stilbella thermophila, from mushroom compost. Stolk (1965) described a new species of Talaromyces isolated from Italian compost viz. T. emersonii, and also emended several previously described thermophilic fungal species. Chang and Hudson (1967a) isolated many of the known thermotolerant and thermophilic fungi from wheat straw compost, and also studied their distribution in great detail. Maheshwari (1968) studied the occurrence of thermophilic fungi in various Indian composts. He isolated several species of thermophilic fungi, confirming their ubiquitous distribution in habitats favouring excessive temperatures, and he concluded that future studies should concentrate on the surface soils and pond waters of the tropics where a thermophilic microflora may exist. Various other workers have examined more unusual composts or plant storage piles. Eggins and Coursey (1964) studied Nigerian oil palm kernel stacks and found that temperatures in the order of 60°C frequently developed. They isolated Chaetomium thermophile, an ascosporic Penicillium sp. (probably Talaromyces emersonii) and a new Thermomyces sp. The latter species was named Thermomyces ibadanesis by

Apinis and Eggins (1966) and found to be a true thermophile, having a minimum temperature for growth of 31-35°C and a maximum of 60-61°C. In fact this is a strongly thermophilic species, similar in taxonomy and temperature relations to Thermomyces lanuginosus. Okafor (1966) recorded temperatures of up to 58°C in Nigerian maize stacks and isolated several thermophilic species from these stacks viz. Thermomyces lanuginosus, Mucor pusillus and a Rhizomucor which he considered to be a new species.

Apinis and Pugh (1967) made an extensive investigation of the thermophilous fungi of birds' nests. They found that the species populations of thermophilous nest fungi were similar to those reported from plant debris on the soil surface and also closely related to the species found in various composts. There was a relatively high frequency of thermophiles suggesting that the nests are warmed up by the birds during incubation and probably by insolation during the summer months, thus creating a habitat conducive to a thermophilic type of growth.

Eveleigh and Brewer (1963) were among the first workers to investigate the occurrence of thermotolerant fungi in industrial wastes. They recorded temperatures of 38-52°C in waste slimes of paper mills in Eastern Canada and isolated a number of thermotolerant fungi

from them. However, only one species, Byssochlamys sp. showed growth at or above 50°C and due to its failure to grow below 25°C it could be considered to be a true thermophile. However, there has been no further record of the fungus. Nilsson (1965) studied birch, spruce, aspen and pine chip piles commonly found in the wood-using mills of Sweden. Chip piles exposed for three to thirteen months showed temperatures of 45-50°C in the pile core. Temperatures above 60°C were also recorded but these sterilised the piles. In the outer parts of the chip piles, where temperatures below 30°C occurred, few thermophilic fungi were recorded. However, the number of thermophiles increased markedly towards the inner regions of high temperature. The thermophilic fungi isolated in order of frequency were: Allescheria terrestris, Penicillium sp., Mucor sp., Sporotrichum thermophile and Thermoascus aurantiacus. Chrysosporium pruinosum, a weakly thermotolerant species, was both the commonest and most damaging fungus in all chip storage. Bergman and Nilsson (1966) continued the study on the chip piles of paper mills combining detailed analyses of the internal conditions of pine chip piles with an investigation of the occurrence and activity of the indigenous micro-fungi. They isolated the thermotolerants

Chrysosporium pruinosum and Aspergillus fumigatus, and five thermophilic species viz. Allescheria terrestris, Chaetomium thermophile, Sporotricum thermophile, Talaromyces emersonii and Thermoascus aurantiacus. Bergman and Nilsson (1967) made further studies on the aspen chip piles of a sulphite mill, using analyses similar to those used previously. They isolated the following thermophilic fungi: Allescheria terrestris, Sporotrichum thermophile, Thermoascus aurantiacus, Cephalosporium sp., Byssochlamys sp., and Dactylomyces thermophilus. The last species was re-isolated for the first time since its initial description by Sopp in 1912. These studies demonstrated that there was a specific thermophilic microflora which initiated or was able to survive in the high temperatures developed in storage chip piles. Essentially this was a very consistent population, qualitatively, being similar in the variety of piles investigated.

The fact that temperature determines the composition of a particular micro-flora has also been shown by Schnathorst and Halisky (1960), who reported that Aspergillus flavus and A. niger seldom appeared on cotton bolls in Central California, where summer temperatures average about 25°C. However, these moulds were widespread

in Southern California, where summer temperatures average 32°C. A similar relationship was recorded earlier by Panasenko (1936), who observed that in the Ukraine species of Aspergillus on cotton bolls were almost absent but in Azerbaidzhan, where summer temperatures were much higher, Aspergillus niger, A. flavus and A. fumigatus were very widespread on cotton bolls. These species are all thermotolerants but obviously require higher temperatures for optimum infection and growth.

The economic importance of thermophilous fungi has been known for some time. Gilman and Barron (1930) found that several thermotolerant Aspergillus species raised the temperature of stored grain to 40-50°C. Christensen and Gordon (1948) investigated the mould flora associated with the heating of moist grain, and a detailed review of the deterioration of stored grain by these fungi has been compiled by Christensen (1957). The moulding of hay, with the subsequent increase of temperature, has been investigated on numerous occasions. More recently Gregory and Lacey (1963) and Gregory et al. (1963) have examined the fungi isolated from self-heating hay in order to correlate the presence of these thermophilous fungi with the occurrence of human and animal diseases ascribed to the inhalation of dust from mouldy

hay. They isolated several of the thermotolerant Aspergilli mentioned above together with the thermophiles Mucor pusillus and Thermomyces lanuginosus. All the spore types were shown to be able to penetrate the deeper parts of the lungs but have not yet been implicated in causing disease symptoms.

Thermophilic fungi and bacteria have also been shown by Fletcher et al. (1967) to occur on the leaves of a tobacco crop. Leaf discs incubated at 50°C showed fungal colonisation on 250 out of a total of 2,500 leaf discs. The micro-organisms isolated were species of Mucor, Streptomyces, Humicola, Myriococcum and Aspergillus. The possible role or economic importance of this specialised microflora, however, was not investigated.

The literature on thermophilic fungi, therefore, is rapidly expanding and their distribution has been shown to be more ubiquitous than was previously thought. The aim of the present investigation was to try and obtain a general picture of the occurrence and distribution of thermophilic fungal populations. It was hoped to achieve this by sampling a variety of natural habitats in order to ascertain if there were any ecological differences in these populations, and then to compare them qualitatively and

quantitatively with semi-natural habitats where the high temperatures generated by microbial activities might favour the growth of thermophiles. It was also decided to investigate artificial habitats such as industrial wastes, etc., which, because of elevated temperatures might induce thermophilic fungal colonisation or perhaps lead to thermal adaptation and so create a characteristic fungal population.

Initially, the aim was to study exclusively the true thermophilic micro-flora and for this reason the isolation plates were incubated at 48-50°C, a relatively high isolation temperature compared with that used by previous workers. However, due to the fact that a substantial number of thermotolerant fungi grew at these temperatures, and obviously were part of this specialised high-temperature group, plus the fact that many of them appeared to be interesting species taxonomically, they were included as an integral part of this investigation. The thermotolerants form an interesting group and bridge the gap between true mesophiles and true thermophiles, showing how adaptation to the specialised thermophilic state could have taken place. Therefore, for these reasons, the general scope of the work was widened to include all those species which appeared on soil isolation plates at temperatures above 45°C, and hence

the study has embraced thermotolerants as well as the true thermophiles.

The qualitative and quantitative results from all the habitats studied are presented in Chapter II.

It became necessary to determine accurately the temperature relations of all the isolated fungi and thus to label them as thermotolerant or thermophilic micro-organisms. The results of this study are presented in Chapter IV.

A seasonal study was undertaken and for a period of twelve months a twice monthly assay was made of one selected habitat together with a soil horizon investigation. The air spora was sampled almost daily over a twelve month period and the two seasonal data were compared to see if any correlation exists between soil and air populations of thermophilous fungi.

All the unusual species or strains are described in Chapter V. The isolation of these species made it necessary to blend together the ecological and taxonomic aspects of thermophilous fungi, whereas in fact, the initial idea was to concentrate mainly on the occurrence and distribution of this interesting group of fungi.

The cellulolytic ability of a number of the species

isolated was determined, primarily in order to discover what part they play in general plant decomposition; justification for the inclusion of this work will be discussed more fully (see Chapter VI).

N.B. The names of the organisms are listed as given by the original authors. Subsequent changes, as well as recently proposed emendations, will be discussed in Chapter V.

C H A P T E R I

M E T H O D S E M P L O Y E D

i. Collection of samples

The samples were collected in 1 oz. glass or polypropylene collecting bottles, previously sterilised by autoclaving at twenty pounds pressure for twenty minutes. When taking soil samples the overlying vegetation was cleared away using a sterilised knife and the collecting bottle was plunged into the substratum and quickly sealed. A sterile spatula and knife were used to assist in the collection of difficult materials, the instruments being flamed in 95% alcohol after each sample. In all cases the samples were quickly transported to the laboratory and examined the same day.

ii. pH

pH readings were taken in the laboratory using a portable glass electrode pH meter. Two gms. of the sample were thoroughly mixed in twenty-five mls. of tap water and left for thirty minutes before measuring. The pH meter was standardised against a buffer solution before and after each sample measurement.

iii. Methods of isolation

The main method used was that described by Warcup (1950) This soil plate method is very useful because of its

simplicity and hence was extremely advantageous in this study when a large number of samples were analysed at any one time. Before plating out, the samples were oven dried at 55°C for five to six hours in sterilised aluminium tins. The original method was modified slightly in order to obtain good colony counts, bearing in mind the low frequencies of thermophilic* fungi in the soil. Waksman et al. (1939a) reported that thermophilous* fungi represented less than 100 per gram of oven dry soil in cultivated soils. Apinis (1963a) suggested that these fungi never exceed 200 per gram in an alluvial soil examined near Nottingham. Preliminary tests were made in order to determine the most suitable amount of soil to use when preparing soil plates. It was finally decided to use a standard weight of 0.5 gms. of oven dry soil per plate, in contrast to the 0.005 - 0.01 gms. suggested by Warcup. The soil was weighed in sterile containers and then sprinkled over the surface of the molten agar, previously cooled to 50-60°C. The plate was then gently swirled to facilitate uniform distribution of the soil particles.

* The concept of 'thermophilic' and 'thermophilous' fungi will be discussed in Chapter IV.

In order to control bacterial growth, which initially heavily infected the plates, streptomycin sulphate (0.04 mgms/ml. of agar) and benzyl penicillin (0.02 mgms/ml. of agar) were added to the cooled medium.

More difficult materials (plant remains etc.) were macerated slightly before being plated out but essentially were treated the same as soil samples, i.e. oven dried and accurately weighed.

The soil plates were incubated at 48-50°C but also occasionally at slightly lower temperatures for comparative reasons. Previous workers (Crisan 1959, Cooney and Emerson 1964) had reported on the problem of the drying out of the agar encountered during incubation at elevated temperatures. However, by pouring deep agar plates (ca. 40-50 ccs. agar/plate) these problems were mostly eliminated. This may also be due to the transition from glass to plastic petri dishes which also greatly reduced the risks of contamination, initially encountered. The loose-fitting lids of the glass petri dishes encouraged water loss and allowed easy access to airborne spores. Another advantage of using plastic petri dishes was that the daily growth of the organisms could be viewed directly using a steroscopic dissecting microscope, without

removing the lid.

From comparative analyses of isolations employing a variety of media, potato dextrose agar was selected to be the principal isolating medium. Yeast-starch agar, Malt extract agar, Cornmeal agar, Sabourard dextrose agar, Oxgall agar and Czapek-Dox agar were also tested but found to favour specific groups of fungi, whereas potato dextrose agar was found to be a good general medium. The other media mentioned were used extensively during subculturing for purposes of identification.

A direct isolation method from the sampled substrate was also tried. The fresh samples were incubated in crystallising dishes at 50°C for two days and then examined. However, it was found necessary to add a quantity of sterile water to each sample before and sometimes during incubation in order to prevent excessive desiccation. Individual colonies or sporing heads were removed with the aid of a fine needle and a dissecting microscope. Unfortunately this method was extremely laborious, especially for a large number of samples, and also liable to excessive contamination. This method was, therefore, used chiefly for cumbersome materials, e.g. wood, leaves, etc.

iv. Subculturing

After two days, or sometimes earlier, the plates were examined for colony growth. Fast growing species often dominated the plates and made subculturing very difficult. However, by careful examination under a stereoscopic dissecting microscope, individual colonies could be picked off using a fine tungsten needle. Often when colonies became overgrown 'streak plates' were made. This involved streaking the desired colony plus possible contaminants several times along the length of an agar plate. By careful examination twelve to twenty-four hours later the desired species in the form of germinating spores could usually be secured with the aid of a fine needle.

Monocultures were usually incubated at 40-45°C where they could be kept for two to four weeks prior to examination. Permanent cultures were prepared using 1 oz. McCartney bottles and 12 oz. standard medical flats with potato dextrose agar as the principal growth medium. Subcultures were made approximately every six months as the longevity of some of the species was suspect.

v. Single-spore isolations

Spores of the fungus to be isolated were transferred with a fine needle from a gross culture to a tube of cooled,

melted, sterile agar which was then gently shaken. A loop of this suspension was transferred to a second agar tube, and the contents of the tube were poured into a plastic petri plate. The plate was incubated for approximately twelve hours and then examined under the dissecting microscope, individual sporelings were removed by means of a fine needle and transferred to sterile agar slants.

vi. Examination of cultures

Cultures could be examined directly under the low-power objective. This enabled the study of delicate species which were difficult to examine using conventional slide mounts. The latter technique sometimes failed to give a true picture of the sporing process and this had to be supplemented by direct examination. However, most morphological studies were made from material mounted on slides in lactophenol and cotton blue.

C H A P T E R I I

A R E A S I N V E S T I G A T E D

The areas investigated can be divided into three sections:

I. Natural areas

II. Semi-natural areas

III. Industrial areas

A range of natural habitats, defined ecologically, was inspected in order to give a general view of the distribution of thermotolerant and thermophilic fungi. These can be considered to be areas in which elevated temperatures are rarely, if ever, encountered, and hence are not particularly favourable to thermophilic growth. The term 'semi-natural' is applied to areas such as compost heaps, birds' nests, etc., where animal agents have created a special environment. These habitats are considered to be conducive to thermophilic micro-organisms due to the abnormally high temperatures created by humification and composting action.

Industrial areas are defined as completely artifical habitats created by modern industry. They were selected as the possible source of a thermophilic micro-flora, because of the high temperatures involved either during the process or as a result of the combustion of waste

products.

From each soil sample four plates were usually made, the soil being selected at random.

Results for each species are expressed as: Number of Isolations, Percentage Frequency of Isolation and Percentage Occurrence.

The number of species isolated includes both varieties and/or unusual strains considered to be of a varietal status.

N.B.

No. of isolations = total no. of colonies of a given species

% frequency of isolation =

$$\frac{\text{total number of isolations of a given species}}{\text{total number of isolations of all species}} \times 100$$

% occurrence =

$$\frac{\text{number of plates on which a given species occurs}}{\text{total number of plates}} \times 100$$

I NATURAL AREAS

a. Grassland

i. Limestone grassland:

Dovedale, Derbys. Sampled June 1967.

ii. Chalk grassland:

Louth, Lincs. Sampled July 1967.

The dominant plant species on chalk grassland are: *Avena pratensis*, *Festuca ovina* and *F. rubra*. *Cirsium acaule*, *Lotus corniculatus* and *Plantago lanceolata* occur abundantly.

The dominant plant species on limestone grassland are: *Festuca ovina* and *Agrostis* spp., whilst abundant species are represented by *Galium verum*, *Lotus corniculatus*, *Poterium sanguisorba* and *Ranunculus bulbosus*.

	Air Temperature °C	Soil Temperature °C	Soil pH
Dovedale	22	20.5	7.2-7.3
Louth	20	19	7.3-7.5

Ten samples were taken from each area to give a total of forty plates.

Results and Discussion

The results are presented in Table 1. The total number of isolations recorded from limestone grassland (area i.) was 442 (11.5 isolates/plate), whilst from chalk grassland (area ii.) was 271 (6.8 isolates/plate). The dominant species isolated from area i. were Aspergillus fumigatus, comprising 46.8% of the total isolations, and Thermomyces lanuginosus, comprising 38.2% of the total isolations. Mucor pusillus constituted only 9.3% of the total isolations but had an occurrence of 70%, i.e. a small number of isolates/plate but occurring on a large number of plates. Similarly, the dominant species in area ii. were A. fumigatus and T. lanuginosus, but at a reduced level comprising 36.2% and 21.0% respectively of the total isolations. This was also reflected in the occurrence of these species: A. fumigatus had a 90% occurrence in area i. compared with 52.5% for area ii., whilst T. lanuginosus had a 97.5% occurrence in the former area but only 40% in the latter. The only other species common to both areas were M. pusillus and Chrysosporium sp., the latter species was one of the most frequently represented of the minority species. Another interesting species, Cephalosporium sp., was

Table 1

Species present in grassland soils

Species	Dovedale			Louth		
	no. isols.	% fr. isol.	% occ.	no. isols.	% fr. isol.	% occ.
Mucor pusillus	41	9.3	70	19	7.0	25
Allescheria terrestris	-	-	-	18	6.6	22.5
Chaetomium thermophile var coprophile	-	-	-	11	4.0	10
Chaetomium thermophile var thermophile	-	-	-	21	7.8	15
Myriococcum albomyces	3	0.7	5	-	-	-
Thermoascus crustaceus	-	-	-	5	1.9	7.5
Aspergillus fumigatus	207	46.8	90	98	36.2	52.5
Cephalosporium sp.	-	-	-	9	3.3	7.5
Chrysosporium sp.	19	4.3	17.5	28	10.3	22.5
Humicola insolens	3	0.7	2.5	-	-	-
Thermoidium sulphureum	-	-	-	5	1.9	5
Thermomyces lanuginosus	169	38.2	97.5	57	21.0	40
Total isolations	442			271		
Number of species	6			10		

infrequently isolated, exclusively from area ii.

The significantly higher number of isolations recorded from limestone grassland compared with that from chalk grassland can be explained by the high isolate counts recorded for A. fumigatus and T. lanuginosus in the former area. Furthermore, although there was a larger number of isolates obtained from limestone grassland it represented only six species, whereas ten species were recorded from chalk grassland. The high proportion of A. fumigatus and T. lanuginosus on each soil plate from chalk grassland may have led to the exclusion of the slower growing species thus reducing the species count on these plates. Although these areas have similar high plant colonisers and soil conditions (e.g. pH), their geographical separation will tend to delimit the microbial populations and may lead to an individual pattern of fungal colonisation.

b. Sand Dune Systems

		Sampled	pH
i.	Borth, Cardiganshire, N. Wales.	February 1967	7.3-7.6
ii.	Abergele, Denbighshire, N. Wales.	April 1968	8.2-8.5
iii.	Freshfield, Lancashire.	May 1968	7.6-7.9

The dune systems at Borth and Freshfield are both very extensive but the flora of all the areas is essentially similar. Ammophilia arenaria is the dominant coloniser with scattered patches of Agropyron junceiforme and Sedum acre. Ten samples were taken to give a total of forty plates for each area. (See Table 2)

Results and Discussion

The results are given in Table 2. The total number of isolations recorded from area i. was 307 (7.7 isolates/plate), from area ii. was 268 (6.7 isolates/plate) and from area iii. was 172 (4.3 isolates/plate). The dominant species in all areas were Aspergillus fumigatus and Thermomyces lanuginosus, these species accounting for over 70% of the total isolations in each area. The percentage occurrence was also significantly higher than any of the other species isolated. The above

Table 2

Species present in sand dune systems

Species	i. Borth			ii. Abergele			iii. Freshfield		
	no. isols.	% fr. isol.	% occ.	no. isols.	% fr. isol.	% occ.	no. isols.	% fr. isol.	% occ.
Absidia ramosa	-	-	-	5	1.9	5	-	-	-
Mucor pusillus	41	13.3	50	-	-	-	17	9.9	20
Mucor sp.	-	-	-	-	-	-	7	4.1	10
Chaetomium thermophile var. dissitum	21	6.8	10	-	-	-	-	-	-
Myriococcum albomyces	9	2.9	15	29	10.9	40	4	2.3	10
Talaromyces duponti	-	-	-	5	1.9	10	-	-	-
Aspergillus fumigatus	119	38.3	80	46	17.2	65	30	17.4	40
Chrysosporium sp.	-	-	-	-	-	-	10	5.8	15
Sporotrichum thermophile	19	6.2	25	-	-	-	-	-	-
Thermoidium sulphureum	5	1.6	10	-	-	-	-	-	-
Thermomyces lanuginosus	93	30.3	85	183	68.2	100	104	60.5	85
Total isolations	307			268			172		
Number of species	7			5			6		

two species, together with Myriococcum albomyces, were the only ones common to all three areas. There was, however, no significant difference in the number of species isolated from each area, ranging from five to seven. The varying number of total isolations from each area can again be accounted for by the fluctuations in the number of isolates of A. fumigatus and T. lanuginosus recorded. The range of pH also varied for each area, specifically high in area ii., but it would appear to have no specific effect on the number of isolates recorded, although the number of species isolated from area ii. was the lowest obtained.

Sand, as a substrate is generally considered to be poor in humus and nutrients, the fact that the samples were taken from around the patches of vegetation may have led to an increase in the number of isolates recorded, compared with bare or poorly colonised sand. Sand, as a conductor of heat is very inefficient and high surface temperatures commonly occur during the summer months due to solar insolation (Vaartaja 1954, Salisbury 1952 p.192). The latter author stated that on a hot summer's day the surface sand may attain temperatures of over 60°C and temperatures of over 40°C have been recorded in the

surface layers during spring. These high temperatures would tend to favour the growth of thermophilous fungi and may lead to increases in the numbers of these species during these months. However, the effects of solar insolation are not sustained for long periods and the populations of thermophilous fungi may "flare up" during the periods of suitable growth conditions. This would lead to an ephemeral life cycle, the individual fungus surviving during unfavourable conditions as a very slow growing or dormant mycelium or producing resting bodies, etc. The apparently ubiquitous nature of Myriococcum albomyces in sand from a wide geographical area may be due to its ability to exploit these ephemeral conditions and then to produce a resistant perithecial stage, able to survive adverse conditions. Both A. fumigatus and T. lanuginosus produce large numbers of spores (asexual), a proportion of which may survive the winter conditions and germinate following the advent of favourable conditions.

c. Marshland

		Sampled	pH
i.	Neston, Wirral	June 1967	7.7-8.0
ii.	Freshfield, Lancs.	April 1968	7.3-7.5

Neston is a fairly extensive salt marsh on the Dee Estuary with a typical salt marsh flora. The dominant plant species are: Armeria maritima, Plantago maritima and Aster tripolium. The marsh at Freshfield is situated immediately behind the grey dunes but is mainly fresh-water in origin. The flora comprises chiefly Salix repens, Hippophaë rhamnoides (sea buckthorn) and Sphagnum species.

Ten samples were taken to give a total of forty plates for each area. (See Table 3)

Results and Discussion

The results are presented in Table 3. The total number of isolations recorded from area i. was 588 (14.7 isolations/plate) and from area ii. was 310 (7.8 isolations/plate). Six species were isolated from each area and four of these were common to both. Mucor pusillus and Thermomyces lanuginosus were the dominant species in area i. comprising 27.4% and 29.2% respectively

Table 3

Species present in marshland

Species	Neston			Freshfield		
	no. isols.	% fr. isol.	% occ.	no. isols.	% fr. isol.	% occ.
Mucor pusillus	161	27.4	95	59	19.0	75
Mucor sp.	-	-	-	21	6.8	20
Chaetomium thermophile var coprophile	20	3.4	15	27	8.7	20
Chaetomium thermophile var dissitum	106	18.0	60	-	-	-
Dactylomyces crustaceus	-	-	-	3	1.0	5
Thermoascus aurantiacus	32	5.4	30	-	-	-
Aspergillus fumigatus	97	16.7	65	37	11.9	30
Thermomyces lanuginosus	172	29.2	100	163	52.6	100
Total isolations	588			310		
Number of species	6			6		

of the total isolations. The latter species was also dominant in area ii. accounting for over half the total isolations. Similarly, M. pusillus was well represented in area ii. and for both areas the occurrence of this species was extremely high, i.e. 95% in area i. and 75% in area ii. Chaetomium thermophile occurred in both areas, and one variety (var. dissitum) was isolated in substantial amounts exclusively from area i. In general, however, the areas had qualitatively similar thermophilous mycofloras, but quantitatively, were significantly different.

Several factors may contribute to the quantitative differences between the two areas. The samples from the salt marsh (area i.) were taken, primarily, from the areas of dense vegetation, i.e. high up the marsh. These areas are flooded irregularly by the sea but large amounts of organic matter accumulate in this region. There would, therefore, be adequate supplies of organic matter present and also few periods when flooding would occur which could lead to anaerobic conditions in the mud layers. The upper communities of the marsh are exposed to a long uninterrupted period of exposure and excessive drying and high temperatures are known to occur in the

surface soil of this region. This may favour the growth of those thermophilous fungi which are able to withstand the alkalinity and salt content of these soils. Elliot (1930) investigated the soil fungi of the Dovey salt marshes and found that the highest counts were obtained during the summer months and an average temperature of 19.5°C was recorded. Fewer fungi were recorded from a gram of salt marsh soil than from one gram of cultivated soil. However, the present results reflect no general shortage of isolates in this habitat, relative to the other areas studied. Pugh (1962) reported that Aspergillus fumigatus was one of the most frequently isolated fungi from a salt marsh investigated at Gibraltar Point, Lincs. This species was found to be more prevalent in the bare mud regions than in the vegetation zones, being of lowest occurrence in the top of the marsh. In the present study A. fumigatus was substantially recorded but was not one of the dominant species.

The marsh studied at Freshfield is non-tidal and is known to be flooded for most of the year, except during the summer months. The long periods of anaerobic conditions may reduce the numbers of isolates of thermophilous species present but these may escalate rapidly

during periods of exposure when the possible occurrence of high surface temperatures, especially in the dense organic matter present, may favour their growth.

d. Pine woods

		Sampled	pH
i.	Peckforton Hills, Cheshire	June 1967 Oct. 1968	3.5-4.6
ii.	Betswy-coed, N. Wales	April 1967 Oct. 1968	5.3-5.9
iii.	Freshfield, Lancs.	April 1968	5.2-6.0
iv.	Delamere Forest, Cheshire	Oct. 1968	3.2-4.5

The pine woods (Pinus sylvestris) at Peckforton and Betswy-coed were sampled on two separate occasions, the other areas being sampled once only. The plantation at Peckforton consists of old trees (80-100 years old) and saplings, and is established on a system of sandstone ridges.

At Betswy-coed afforestation of the upper moorlands (up to 1000 ft.) has been progressing for a number of years. Old and new plantations were sampled, the former with virtually no ground flora whilst the latter has a typical moorland flora.

Afforestation at Freshfield began about 40-50 years ago and is based primarily on old sand dunes. A podzolised soil has been formed under the cover of Pinus nigra laricio and in most areas there is an

exceptionally dense pine litter with little or no ground flora.

Delamere Forest is situated on glacial sands and planting began about one hundred years ago. A well developed podzol has been formed under Pinus sylvestris, the present trees being about sixty to eighty years old.

Ten samples were taken from Peckforton and Betswy-coed, to give a total of forty plates.

Five samples were taken from Freshfield and Delamere to give a total of twenty plates.

Results and Discussion

The results are presented in Tables 4 and 5. The total number of isolations recorded from area i. was 439 (11.0 isolates/plate), from area ii. was 131 (3.3 isolates/plate), from area iii. was 160 (8.0 isolates/plate) and from area iv. was 162 (8.1 isolates/plate). Ten species were isolated from area i., six species from area ii. and seven from areas iii. and iv. Four species were found to occur in the four areas studied, viz: Aspergillus fumigatus, Mucor pusillus, Thermoascus aurantiacus and Thermomyces lanuginosus. A fumigatus was dominant in all these areas, representing

Table 4

Species present in pine woods

Species	i. Peckforton			ii. Betswy-coed		
	no. isols.	% fr. isol.	% occ.	no. isols.	% fr. isol.	% occ.
Mucor pusillus	74	16.8	60	16	12.2	22.5
Mucor sp.	2	0.4	2.5	-	-	-
Allescheria terrestris	5	1.1	7.5	-	-	-
Dactylomyces crustaceus	6	1.4	5	-	-	-
Talaromyces duponti	3	0.7	5	2	1.5	2.5
Talaromyces emersonii	53	12.1	55	15	11.5	20
Thermoascus aurantiacus	49	11.2	35	10	7.6	10
Aspergillus fumigatus	179	40.8	65	68	51.9	52.5
Aspergillus fumigatus (orange var.)	18	4.1	10	-	-	-
Thermomyces lanuginosus	50	11.4	55	20	15.3	25
Total isolations	439			131		
Number of species	10			6		

Table 5

Species present in pine woods

Species	iii. Freshfield			iv. Delamere		
	no. isols.	% fr. isol.	% occ.	no. isols.	% fr. isol.	% occ.
Absidia ramosa	10	6.2	25	-	-	-
Mucor pusillus	42	26.3	85	10	6.2	25
Mucor sp.	-	-	-	5	3.1	20
Rhizopus sp. 3	3	1.9	5	-	-	-
Chaetomium thermophile var. thermophile	4	2.5	10	-	-	-
Dactylomyces crustaceus	-	-	-	8	4.9	15
Talaromyces emersonii	-	-	-	11	6.8	30
Thermoascus aurantiacus	9	5.6	20	29	17.9	50
Aspergillus fumigatus	76	47.5	100	93	57.4	70
Thermomyces lanuginosus	16	10.0	35	6	3.7	15
Total isolations	160			162		
Number of species	7			7		

approximately half the total isolates of each area. Talaromyces emersonii was recorded from three of the four and was well represented in areas i. and ii., particularly in area i. with an occurrence of 55%.

The relatively high number of isolates recorded from area i. may be due, in part to the open nature of these pine woods. The other areas studied, particularly areas ii. and iii., have an exceptionally dense stand of pine, and solar insolation during the summer months therefore, will be negligible. There may be, in these cases, relatively few periods when soil temperatures are high enough to support the growth of thermophiles. The exceptional differences between the numbers of isolates from areas i. and ii. may be further explained by their geographical locations. The pine woods in area i. are situated on a sandstone outcrop overlooking the Cheshire plain and it may function in this respect as a giant spore trap, hence accounting for the large numbers of isolates and wide range of species recorded. The pine woods in area ii. are situated at a high altitude and unfavourable conditions may persist for long periods of the year. Hence, growth of the thermophilous mycoflora will be restricted and it is interesting to note that

over half the total species isolated are capable of producing resistant overwintering structures.

Parkinson and Balasooriya (1967) found that the soils of Freshfield (area iii.) and Delamere (area iv.) were very similar in their mycofloras. They concluded that the existence of such similar populations in habitats so widely separated illustrates the profound influence of vegetation cover on soil mycofloras. The present results suggest some similarities in the thermophilous mycofloras of these areas. Almost identical numbers of isolates and species were recorded, but small qualitative differences were obvious. In general, there are similarities in the thermophilous mycoflora of all these areas but the physical nature of the pine wood and the geographical location may have considerable effects on this mycoflora, e.g. compare areas i. and ii.

e. Mixed deciduous woodland

		Sampled	pH
i.	Peckforton Hills, Cheshire	Oct. 1968	3.9-4.4
ii.	Delamere, Cheshire	Oct. 1968	4.9-5.1

The woodland of both areas consisted mainly of Quercus and Betula species. A rich humus layer was present together with a scattered flora of Dryopteris and a varied moss cover.

Five samples were taken to give a total of twenty plates.

Results and Discussion

The results are presented in Table 6. The total number of isolations recorded from area i. was 372 (18.6 isolates/plate) and from area ii. was 64 (3.2 isolates/plate). The main discrepancy in the numbers of isolates can be correlated with the absence of Aspergillus fumigatus from area ii., whereas in area i. the frequency of isolation of this species was 51.9%, (100% occurrence), and was by far the most prevalent species recorded. The dominant species in area ii. were Mucor pusillus, 32.8% of the total isolations, and Thermoascus aurantiacus, 35.9% of the total isolations.

Table 6

Species present in mixed deciduous woodland

Species	Peckforton			Delamere		
	no. isols.	% fr. isol.	% occ.	no. isols.	% fr. isol.	% occ.
Mucor pusillus	45	12.1	90	21	32.8	60
Mucor sp.	2	0.5	10	5	7.8	20
Talaromyces emersonii	19	5.1	35	8	12.5	25
Thermoascus aurantiacus	56	15.0	85	23	35.9	50
Aspergillus fumigatus	193	51.9	100	-	-	-
Sporotrichum thermophile	6	1.6	10	-	-	-
Thermomyces lanuginosus	51	13.7	75	7	11.0	20
Total isolations	372			64		
Number of species	7			5		

These two species were also frequently recorded from area i. and had a high percentage occurrence on the isolation plates. Furthermore, the five species isolated from area ii. all occurred in area i., and hence there would appear to be certain qualitative similarities between the two areas.

The numerical differences between the two areas is difficult to explain and the obvious predominance of A. fumigatus in area i. enhances these differences. The open woodland of area i. based on well-drained sandstone soil may be more conducive to the thermophilous growth type than the dense woodland of area ii. chiefly occurring around waterlogged ground. The suppositions discussed earlier, for the results obtained from the pine woods of Peckforton, may also apply.

f. General woodland soils

	Sampled	pH
i. *Larix* woodland Keele, Staffs.	May 1968 Aug. 1968	6.0
ii. *Taxus* woodland Keele, Staffs.	May 1968 Aug. 1968	5.8
iii. *Salix* and *Betula* woodland Keele, Staffs.	May 1968 Aug. 1968	6.0
iv. *Fagus* woodland Keele, Staffs.	May 1968 Aug. 1968	5.9
v. *Alnus* swamp Freshfield, Lancs.	April 1968	6.1

The woodland soils in the Keele area were sampled on two separate occasions and involved several different examples of each woodland type.

Results and Discussion

The final results are presented out of a total sample of twenty plates for each woodland type and these are given in Table 7.

The total number of isolations recorded from area i. was 143 (7.1 isolates/plate), from area ii. was 171 (8.5 isolates/plate), from area iii. was 57 (2.8 isolates/plate), from area iv. was 88 (4.4 isolates/plate) and finally from area v. was 298 (14.9 isolates/plate).

Table 7

Species present in general woodland soils

Species	i. Larix			ii. Taxus			iii. Salix and Betula			iv. Fagus			v. Alnus		
	no.	% fr	% occ.	no.	% fr	% occ.	no.	% fr	% occ.	no.	% fr	% occ.	no.	% fr	% occ.
Absidia ramosa	-	-	-	-	-	-	-	-	-	-	-	-	79	26.5	95
Mucor pusillus	11	7.7	25	28	16.4	50	20	35.1	65	9	10.2	20	58	19.5	100
Mucor sp.	3	2.1	20	-	-	-	-	-	-	-	-	-	3	1.0	10
Allescheria terrestris	10	7.9	20	-	-	-	-	-	-	6	6.8	10	-	-	-
Chaetomium thermophile var. dissitum	-	-	-	-	-	-	-	-	-	-	-	-	12	4.0	25
Dactylomyces crustaceus	-	-	-	-	-	-	4	7.0	15	-	-	-	-	-	-
Dactylomyces thermophilus	-	-	-	-	-	-	4	7.0	10	-	-	-	-	-	-
Talaromyces emersonii	-	-	-	8	4.7	15	13	22.8	25	-	-	-	-	-	-
Thermoascus aurantiacus	-	-	-	15	8.8	25	-	-	-	19	21.6	35	-	-	-
Aspergillus fumigatus	-	-	-	36	21.0	45	-	-	-	22	25.0	20	132	44.3	100
Thermomyces lanuginosus	119	83.2	100	84	49.1	85	16	28.1	45	32	36.4	70	14	4.7	40
Total isolations	143			171			57			88			298		
Number of species	4			5			5			5			6		

The numbers of species isolated was relatively small, ranging from four to six, and only two species were common to the five areas, viz: Mucor pusillus and Thermomyces lanuginosus. The latter species formed the bulk of the isolations from areas i.-iv., but was less frequent in area v. In this area, Aspergillus fumigatus had a high frequency of isolation (44.3%) and together with Absidia ramosa (26.5%) and Mucor pusillus (19.5%) represented over 90% of the total isolations. These three species were also present on a large proportion of the plates, i.e. 95-100% occurrence. Besides the species mentioned above, very few others were recorded in any numbers from the areas investigated. Only Talaromyces emersonii (area iii.) and Thermoascus aurantiacus (area iv.) represented significant proportions of the total isolations from one area.

There would appear, therefore, to be qualitative as well as quantitative differences between the thermophilous mycofloras of several woodland types, even in the same general sampling region. The occurrence of Dactylomyces thermophilus, a rarely reported species, from one area only and the limited distribution of other species may be due, in part, to the varied environmental

factors encountered in these habitats. The Alnus swamp, at the time of sampling was almost completely dried up, due to a period of unusually high temperatures, and the swamp litter was particularly dense, and these factors may explain the high isolate counts recorded from this area.

g. Mountainous areas

	Sampled	pH range
i. Betswy-coed, N. Wales	April 1968	5.7-5.9
ii. Loch Torridon, Wester Ross	July 1967	5.2-5.6

Area i. is situated several miles from Betswy-coed at an elevation of about 1000 ft.

Area ii. is situated above Loch Torridon on the sides and near the summit of Liathach (1500-2000 ft.)

In both areas Calluna vulgaris is dominant, giving rise to a very thick litter, with a supporting vegetation of various mosses and lichens.

Ten samples were taken, making a total of forty plates for each area.

Results and Discussion

The results are presented in Table 8. The total number of isolations recorded from area i. was 140 (3.5 isolates/plate) and from area ii. was 166 (4.1 isolates/plate). Five species were isolated from area i. and eight species from area ii, four species were common to both areas, viz: Mucor sp., Thermoascus aurantiacus Aspergillus fumigatus and Thermomyces lanuginosus. A. fumigatus formed a high proportion of the total

Table 8

Species present in mountainous areas

Species	Betswy-coed			Torridon		
	no. isols.	% fr. isol.	% occ.	no. isols.	% fr. isol.	% occ.
Mucor pusillus	19	13.6	25	-	-	-
Mucor sp.	4	2.9	7.5	11	6.6	10
Allescheria terrestris	-	-	-	9	5.4	15
Myriococcum albomyces	-	-	-	3	1.8	7.5
Talaromyces emersonii	-	-	-	4	2.4	5
Thermoascus aurantiacus	3	2.1	5	10	6.0	10
Aspergillus fumigatus	61	43.6	50	43	25.9	37.5
Aspergillus fumigatus (orange var)	-	-	-	65	39.2	52.5
Thermomyces lanuginosus	53	37.8	40	21	12.7	32.5
Total isolations	140			166		
Number of species	5			8		

isolations in area i. (43.6%) and was also well represented in area ii. (25.9% of the total isolations). However, an orange mutant strain of A. fumigatus was particularly prevalent in area ii. and was the dominant species in this area, comprising 39.2% of the total isolations and with a relatively high occurrence (52.5%). In area i. Thermomyces lanuginosus together with A. fumigatus formed the bulk of the isolations. The Ascomycetes, Allescheria terrestris, Myriococcum albomyces and Talaromyces emersonii, were recorded in small amounts from area ii. but were not represented at all in area i.

The ability of thermophilous species to survive in these areas, where warm conditions would be infrequent, must depend on the production of overwintering stages (e.g. Ascomycetes) or the ability of the mycelium or spores to survive the periods of unfavourable conditions. Solar insolation may be significant at these high altitudes during the summer months, especially in the thick Calluna litter which would tend to absorb and hold the heat. The number of species isolated from area ii. is surprisingly high as this area is particularly exposed to the elements, the mountain rises directly from sea-level to over 2000 ft. in a relatively short distance and

little or no tree cover is present except on the lower slopes. The occurrence of a number of Ascomycetes may be due to their ability to survive the winter months, in the form of perithecia etc., and grow during the periods of high solar insolation, which may be significant in this exposed habitat. The high frequency of occurrence of the orange mutant of Aspergillus fumigatus is particularly interesting, due to the fact that a recent paper by Rai et al. (1968) discussed the appearance of two similar mutants of A. fumigatus (buff and tan mutants) isolated for the first time from any natural habitat. These authors imply that the two mutant strains arose as a result of the rigorous physical conditions present in the Indian alkaline soils investigated. A. fumigatus was isolated in abundance from the latter soils and the mutants may have evolved due to the intense solar radiation encountered in these areas. It is interesting to speculate if the same conditions may occur in the present habitat.

II SEMI-NATURAL AREAS

a. Manures and composts

i. Animal droppings: horse, cow, rabbit.

ii. Man-made composts: straw and animal manure, grass cuttings and mushroom compost.

Temperatures of 35-62°C were recorded from mushroom compost and temperatures of 21-53°C recorded for the various manures sampled. The other sampled materials never showed unusually high temperatures although they were often two to three degrees higher than the surrounding environment.

In trial plates it was found necessary to reduce the initial inoculum from 0.5 gms. to 0.1 gms., in order to aid counting and subculturing of the resulting colonies.

Results and Discussion

The results are presented for each type of material from twenty plates, but the straw and animal manure results are derived from forty plates.

The results are given in Tables 9 and 10. In group i., animal droppings, the total number of isolations recorded from horse dung was 361 (15.0 isolates/plate),

Table 9

Species present in various animal droppings

Species	Horse			Cow			Rabbit		
	no. isols.	% fr. isol.	% occ.	no. isols.	% fr. isol.	% occ.	no. isols.	% fr. isol.	% occ.
Mucor pusillus	48	13.3	90	51	26.2	75	29	21.2	55
Rhizopus sp. 1	-	-	-	-	-	-	*4	2.9	5
Rhizopus sp. 2	-	-	-	28	14.4	80	-	-	-
Chaetomium thermophile var. coprophile	58	16.1	50	-	-	-	-	-	-
Chaetomium thermophile var. dissitum	32	8.9	35	15	7.7	65	9	6.6	35
Chaetomium thermophile var. thermophile	24	6.6	10	-	-	-	-	-	-
Aspergillus fumigatus	7	1.9	10	-	-	-	20	14.6	55
Humicola insolens	-	-	-	39	20.0	100	-	-	-
Thermomyces lanuginosus	192	53.2	100	62	31.8	70	75	54.7	70
Total isolations	361			195			137		
Number of species	6			5			5		

* = from sample collected on coal spoil tips

= from 0.1 gms.

from cow dung was 195 (9.7 isolates/plate) and from rabbit dung was 137 (6.9 isolates/plate). The number of species isolated from each type was similar, i.e. five or six. Three species were common to the three substrates, viz: Mucor pusillus, Chaetomium thermophile var. dissitum and Thermomyces lanuginosus. The latter species was also the most frequently isolated in all cases, and occurred on a high percentage of isolation plates. C. thermophile var. coprophile was isolated only from horse dung and occurred in substantial amounts; this species was initially reported by Cooney and Emerson from horse dung. The other variety (var. dissitum) would appear to have a more general distribution in herbivore dung due to its consistent occurrence in the three types of dung investigated.

In general, the relatively limited number of species isolated from these substrates may, in part, be due to the difficulties involved in the colonisation of dung. The samples were taken mainly from fresh droppings and the amount of time which would be available for fungal colonisation would, therefore, be fairly restricted. Furthermore, the actual number of species which had colonised the dung by wind-blown spores etc. cannot be

determined and it may be that a substantial proportion are disseminated by the animal agent. These species would initially be present on the herbage, and ultimately be deposited in the dung, where initially high temperatures would favour their growth and sporulation. A number of thermophilic fungal species have previously been isolated from herbivore dung and these are listed by Cooney and Emerson (1964, p. 123).

In group ii., manures and composts, the total number of isolations recorded from mushroom compost was 346 (17.3 isolates/plate), from grass cuttings was 316 (15.8 isolates/plate) and from straw and animal manures was 760 (19.0 isolates/plate). The number of species isolated varied considerably: five species were recorded from mushroom compost, seven species from grass cuttings and twelve species from straw and animal manures. Only three species were common to the three substrate types, viz: Chaetomium thermophile var. coprophile, Aspergillus fumigatus and Thermomyces lanuginosus. The latter species was again the most frequently recorded and occurred on a very high proportion of the isolation plates (95-100%). In fact, T. lanuginosus was the only species common to all the six types of manure and

Table 10

Species present in composts and manures

Species	Mushroom			Grass			Straw and Animal Manure		
	no. isols.	% fr. isol.	% occ.	no. isols.	% fr. isol.	% occ.	no. isols.	% fr. isol.	% occ.
Absidia ramosa	-	-	-	-	-	-	4	0.5	10
Mucor pusillus	-	-	-	49	15.5	80	56	7.4	55
Chaetomium thermophile var. coprophile	4	1.2	10	23	7.3	40	109	14.3	75
Myriococcum albomyces	-	-	-	-	-	-	8	1.0	10
Talaromyces emersonii	-	-	-	-	-	-	15	2.0	10
Talaromyces duponti	-	-	-	12	3.8	15	19	2.5	12.5
Thermoascus aurantiacus	-	-	-	11	3.5	20	-	-	-
Aspergillus fumigatus	53	15.3	80	104	32.9	90	195	25.7	77.5
Humicola insolens	24	6.9	25	-	-	-	86	11.3	95
Humicola sp. 1	-	-	-	-	-	-	7	0.9	7.5
Humicola sp. 2	-	-	-	-	-	-	10	1.3	10
Sporotrichum thermophile	-	-	-	8	2.5	15	-	-	-
Thermoidium sulphureum	-	-	-	-	-	-	5	0.7	7.5
Thermomyces lanuginosus	162	46.8	100	109	34.5	95	246	32.4	100
Torula thermophila	103	29.8	95	-	-	-	-	-	-
Total isolations	346			316			760		
Number of species	5			7			12		

compost sampled.

Torula thermophila was isolated exclusively from mushroom compost; it constituted a significant proportion of the total isolations (29.8%) and had a 95% occurrence. The specific nature of this compost may be conducive to colonisation by T. thermophila as it was from this source that the initial isolations were made (Cooney and Emerson 1964, p.88). However, this fungus was not isolated by Fergus (1964) during as extensive investigation of this habitat, although he isolated a wider variety of thermophilous fungi than was recorded during the present investigation of mushroom compost. Four of the five species isolated during the present study of mushroom compost were recorded by Fergus, but Thermomyces lanuginosus, notably, was rarely reported by him. The low number of species isolated from mushroom compost in comparison with the numbers from grass cuttings and straw and animal manures, may, in part, have been due to the very high temperatures recorded during sampling. Certain species have been reported to be excluded during the middle stages of composting, when the temperatures are at their near-maximum. Klopotek (1962) studied the occurrence and behaviour of fungi involved in the

composting of municipal refuse. The author recorded seven species of thermophilous fungi from raw material but only three species were isolated during peak composting and the finished or cooled-down compost showed the highest number of species, i.e. twelve. Hormiscium sp., thought by Cooney and Emerson to be Torula thermophila, was one of the species isolated during the middle stages. Also Thermomyces lanuginosus was found to be the predominant fungus during certain stages of composting and this is in good agreement with the present results.

Straw and animal manures would appear to be particularly rich in their range of thermophilous fungi, and this may be due to the wide range of temperature encountered in these habitats. The specific manures which were investigated were in various stages of decomposition and this may account for the variation in temperature. Two interesting Humicola species were recorded in small amounts from straw and animal manure which was undergoing extensive self-heating. Henssen (1957) isolated Aspergillus fumigatus, Thermomyces lanuginosus, Humicola sp. (insolens)and Sporotrichum

thermophile from stable manure undergoing decomposition. The latter species was rarely recorded but the other species occurred in a number of samples. These results correlate well with the present findings and, in particular, the fact that S. thermophile was isolated only from grass cuttings.

b. Sewage

		Sampled	pH range
i.	Working pits	June 1967 Aug. 1968	7.4-7.6
ii.	Derelict pits	June 1967 Aug. 1968	6.6-6.9

Keele University has a small sewage farm which operates on a relatively old-fashioned technique. The untreated sewage is allowed to lie in shallow, cinder-lined, concrete pits. Eventually the entire water content evaporates and the fine textured remains are dumped nearby and gradually incorporated into the soil. This process often takes several months, depending on the weather conditions, and an algal mat is sometimes evident over the sewage surface. During sampling, temperatures of 25-29°C were recorded in these pits and it is supposed that higher temperatures may be developed during the different stages.

A number of derelict pits were also sampled, these had been left for seven years or more without being cleaned out. In this time a dense weed growth of extreme vigour had developed on the rich substratum available and a number of macro-fungi were evident. The object was to

compare the effects of time and vegetation on the numbers and types of thermophilous fungi in the new and old sewage pits.

A sample of twenty-five plates was recorded for each area studied and 0.25 gms was employed as the standard inoculum (not 0.5 gms.).

Results and Discussion

The results are presented in Table 11. The total number of isolations recorded from area i., with no vegetation was 393 (15.7 isolates/plate) and from area ii., vegetation present, was 423 (17.0 isolates/plate). There would appear to be, therefore, no significant quantitative differences between the two areas investigated. The number of species isolated was also similar, being fifteen from area i., and twelve from area ii., and these were also qualitatively similar, as ten species were common to both areas. The warmer temperatures encountered in area i. may be responsible for the slightly higher number of species isolated from that area. In both areas, Thermomyces lanuginosus was the most frequently isolated species and occurred on a high proportion of the isolation plates. Aspergillus fumigatus and Mucor pusillus were substantially recorded from area i. but

Table 11

Species present in sewage material

Species	i. No vegetation			ii. Vegetation present		
	no. isol.	% fr. isol.	% occ.	no. isol.	% fr. isol.	% occ.
Absidia ramosa	2	0.5	4	-	-	-
Mucor pusillus	81	20.6	60	22	5.2	32
Mucor sp.	13	3.3	20	-	-	-
Rhizopus sp. 2	-	-	-	2	0.5	8
Chaetomium thermophile var coprophile	13	3.3	16	56	13.2	40
Chaetomium thermophile var dissitum	25	6.4	20	5	1.2	8
Chaetomium thermophile var thermophile	6	1.5	12	5	1.2	12
Dactylomyces crustaceus	9	2.3	20	2	0.5	8
Myriococcum albomyces	3	0.8	8	-	-	-
Talaromyces duponti	2	0.5	8	6	1.4	12
Talaromyces emersonii	-	-	-	44	10.4	36
Thermoascus aurantiacus	45	11.4	68	103	24.3	92
Aspergillus fumigatus	78	19.8	64	18	4.3	32
Humicola insolens	14	3.6	12	4	0.9	8
Sporotrichum thermophile	3	0.8	12	-	-	-
Thermoidium sulphureum	6	1.5	16	-	-	-
Thermoascus lanuginosus	93	23.7	72	156	36.9	84
Total isolations	393			423		
Number of species	15			12		

very much reduced in area ii. Conversely, Thermoascus aurantiacus was particularly prevalent in the latter area as also was Talaromyces emersonii, which was completely absent from area i. Hence there are individual species differences between the two areas but, in general, the effect of vegetation on the populations of thermophilous fungi was not significant in comparison with those populations present in the areas where vegetation was absent. In the latter area, of course, there would be no shortage of nutrients and the warm surface temperatures might encourage a richer variety of thermophilous fungi.

From town refuse, Klopotek (1962) recorded a number of species which were isolated during the present study. In the stages before extensive decomposition, Aspergillus fumigatus (70%) and Mucor pusillus (20%) were the dominant species isolated. The other 10% included a number of true thermophiles: Penicillium duponti and to a lesser extent Chaetomium thermophile, Thermoascus aurantiacus, Thermoidium sulphureum and Thermomyces lanuginosus. In general, these species were found to occur in substantial amounts during the present investigation of sewage material.

c. Birds' nests

i.	Thrush	10 nests examined
ii.	Blackbird	10 nests examined
iii.	Wren	2 nests examined
iv.	Swallow	2 nests examined

The nests were collected in the summer of 1968 from the Keele area. The Thrush nests were either Song Thrush or Missel Thrush and were lined with a thick mud layer. The Blackbirds' nests were lined with mosses, twigs, grasses and various other debris. The nests of Wren were lined with a dense mat of feathers and mosses providing a well insulated medium. The mud constructed nests of Swallow were lined with numerous feathers.

The nests were dismantled in the laboratory and macerated material from the lining plus fine mineral and organic particles were used to seed the plates. The standard 0.5 gms. of oven dried material was added to each plate.

Results and Discussion

The results of the isolation and occurrence of thermophilous fungi from Blackbird and Thrush nests were

taken from an analysis of forty plates for each species. Those from Wren and Swallow nests were taken from an analysis of ten plates for each species. The complete results are presented in Table 12.

The total number of isolations recorded from Thrush nests was 753 (18.8 isolates/plate), from Blackbird nests was 794 (19.9 isolates/plate), from Wren nests was 108 (10.8 isolates/plate) and from Swallow nests was 146 (14.6 isolates/plate). The thermophilous fungal population of Thrush and Blackbird nests appeared to be similar. Twelve species were isolated from the former nests and nine species from the latter; eight of these species were common to both nest types. There are, therefore, quantitative and qualitative similarities between the nests of these two bird species. This was also reflected in the dominant species recorded: Aspergillus fumigatus and Thermomyces lanuginosus had the highest frequency of isolation in both nest types. Furthermore, Mucor pusillus and Chaetomium thermophile were well represented in the nests of these two bird species.

The isolations from Wren and Swallow nests were numerically similar and the same number of species were

Table 12

Species present in birds' nests

Species	Thrush			Blackbird			Wren			Swallow		
	no. isol.	% fr. isol.	% occ.	no. isol.	% fr. isol.	% occ.	no. isol.	% fr. isol.	% occ.	no. isol.	% fr. isol.	% occ.
Absidia ramosa	31	4.1	10	-	-	-	-	-	-	-	-	-
Mucor pusillus	126	16.7	87.5	76	9.6	35	8	7.4	30	7	4.8	30
Mucor sp.	-	-	-	-	-	-	-	-	-	17	11.6	40
Rhizopus sp. 3	6	0.8	7.5	41	5.2	17.5	-	-	-	-	-	-
Aspergillus nidulans	4	0.5	7.5	-	-	-	-	-	-	-	-	-
Chaetomium thermophile var. coprophile	86	11.4	67.5	110	13.8	45	4	3.7	30	43	29.4	70
Chaetomium thermophile var. dissitum	25	3.3	20	31	3.9	20	-	-	-	4	2.7	10
Talaromyces duponti	-	-	-	-	-	-	4	3.7	20	-	-	-
Talaromyces emersonii	-	-	-	5	0.6	5	5	4.6	20	-	-	-
Thermoascus aurantiacus	53	7.0	35	22	2.8	22.5	-	-	-	-	-	-
Aspergillus fumigatus	172	22.8	70	190	23.9	47.5	31	28.7	40	9	6.2	20
Humicola insolens	16	2.1	12.5	25	3.1	17.5	-	-	-	36	24.7	60
Sporotrichum thermophile	19	2.5	10	-	-	-	3	2.8	30	-	-	-
Thermomyces lanuginosus	210	27.9	100	294	37.0	77.5	53	49.1	100	30	20.5	40
Torula thermophila	5	0.7	5	-	-	-	-	-	-	-	-	-
Total isolations	753			794			108			146		
Number of species	12			9			7			7		

present. However, there were definite differences in the types of species recorded. Four species were common to both Wren and Swallow nests, viz: M. pusillus, C. thermophile var. coprophile, A. fumigatus and T. lanuginosus, and in fact, these were the only species common to all four nest types. Furthermore, the latter species represented by far the highest proportion of the total isolations for Thrush, Blackbird and Wren nests. It also formed a substantial part of the isolates recorded from Swallow nests. In the latter nests, C. thermophile var. coprophile and Humicola insolens were particularly abundant and represented more than half the total isolations. The greater number of nests of Blackbird and Thrush which were examined, when compared with the other types, may account for the richer thermophilous mycoflora recorded. But, in general, it would appear that the former nests are richer in the number of isolates and species of thermophilous fungi in comparison with the nests of Wren and Swallow.

Apinis and Pugh (1967) isolated a wider variety of thermophilous fungal species from birds' nests and this can be accounted for, in part, by the lower incubation temperatures employed during isolation. Indeed, they

noted the disappearance of some of these species at incubation temperatures of 45°C. There is some correlation between their results and the present findings. For example, nine of the species isolated by Apinis and Pugh were also recorded from birds' nests during the present study. However, there are some notable differences: Allescheria terrestris, Chaetomium thermophile and Thermoidium sulphureum are described by the authors as "common nest fungi", but the present study isolated only C. thermophile (in abundance) and the other two species were not recorded. The thermophiles, Talaromyces emersonii and Thermoascus aurantiacus were isolated from two species of birds' nests during the present investigation but were not listed by the above authors. Nevertheless, the abundance of A. fumigatus, C. thermophile and T. lanuginosus in birds' nests is evident from a comparison of the two sets of results, and can be similarly compared with those from other semi-natural areas.

a. Power stations

Meaford power station supplies most of the electricity to the Stoke-on-Trent complex and is situated between Newcastle-under-Lyme and Stone, Staffs.

The huge concrete cooling towers are taken out of operation annually for cleaning purposes and it was on these occasions that sampling was carried out. Two towers were sampled, cooling tower I in May 1967 and cooling tower II in August 1968. The base of the interior of each tower is covered by a parallel arrangement of wooden slats, several layers deep. During working conditions these slats are continually bathed in steam, thus creating a warm and very humid atmosphere. Both towers showed extensive grey mud deposits on the slats, supporting a fairly luxuriant algal and moss growth.

pH varied from 7.6 to 7.9.

Five random samples were taken from each tower, including decaying wood as well as the mud deposits, to give a total of twenty plates per tower.

Results and Discussion

All the plates were covered in less than twenty-four hours and presumably most colonies arose from actively growing mycelium present in the material.

The results are presented in Table 13. The total number of isolations recorded from tower I was 179 (9.0 isolates/plate) and from tower II was 428 (21.4 isolates/plate). Ten species were isolated from tower I and only five species from tower II. Four species were common to both towers. The results, therefore, are qualitatively and quantitatively extremely varied, tower I being distinguishable by having a small number of isolates represented by a wide range of species, whereas tower II had a large number of isolates composed of a few species.

Aspergillus fumigatus was by far the most abundant species isolated from tower I, accounting for 43% of the total isolations, but was completely absent from tower II. In this tower, Thermomyces lanuginosus was the commonest species isolated, 45.1% of the total isolations, with Mucor pusillus and Chaetomium thermophile var. coprophile being well represented. These three species were present

Table 13

Species present in cooling towers

Species	Tower I			Tower II		
	no. isols.	% fr. isol.	% occ.	no. isols.	% fr. isol.	% occ.
Absidia corymbifera	3	1.7	10	-	-	-
Absidia ramosa	8	4.5	15	21	4.9	20
Mucor pusillus	52	29.0	70	119	27.8	100
Rhizopus sp. 3	9	5.0	15	-	-	-
Allescheria terrestris	5	2.8	10	-	-	-
Chaetomium thermophile var coprophile	7	3.9	15	89	20.8	100
Chaetomium thermophile var dissitum	-	-	-	6	1.4	15
Aspergillus fumigatus	77	43.0	90	-	-	-
Chrysosporium sp.	2	1.1	5	-	-	-
Sporotrichum thermophile	11	6.2	20	-	-	-
Thermomyces lanuginosus	5	2.8	10	193	45.1	100
Total isolations	179			428		
Number of species	10			5		

on all the isolation plates analysed from tower II, i.e. 100% occurrence. M. pusillus was also frequently isolated from tower I, but the remaining species were recorded in very small amounts.

A tentative suggestion to explain these widely conflicting results involves assessing the internal conditions likely to be present in these towers. During the time that the towers are in active use, considerable temperature fluctuations are known to exist between the various towers. When slack periods (i.e. reduced power demand) occur, some of the towers may be shut down or not fully exploited and hence temperature gradients may be created. The species isolated from tower II are almost exclusively true thermophiles, and can therefore grow at and withstand consistently higher temperatures than can the thermotolerants, the latter being well represented in tower I. This explanation is based on supposition when related to the two towers investigated, as no accurate information on the temperatures which exist in any one specific tower is available. The small number of species isolated from tower II may be due to consistently high temperatures occurring in this tower so limiting the number of species able to colonise this

habitat, thus reducing competition and resulting in a large number of isolates of those species which can grow under optimum conditions. Conversely, the large number of isolates of fast growing species, e.g. M. pusillus and C. thermophile may have obscured the slower growing species on the initial isolation plates from tower II.

Owing to the high proportion of lignin and cellulose (wooden slats) which is found in these habitats, an ability to utilise these materials may be an added advantage to their colonisation by thermophilous fungi. Bergman and Nilsson (1966) found that Allescheria terrestris and Sporotrichum thermophile could cause wood decay. Each species was found to cause a weight loss of 4% after two months decay of pine sapwood blocks at 50°C. However, these species do not represent a high proportion of the isolates recorded, being present in small amounts.

Nevertheless, it would appear from the results that a range of thermophilous fungi, as well as higher plants, have adapted to the unusual conditions present in the cooling towers of power stations.

b. Coal Spoil Tips

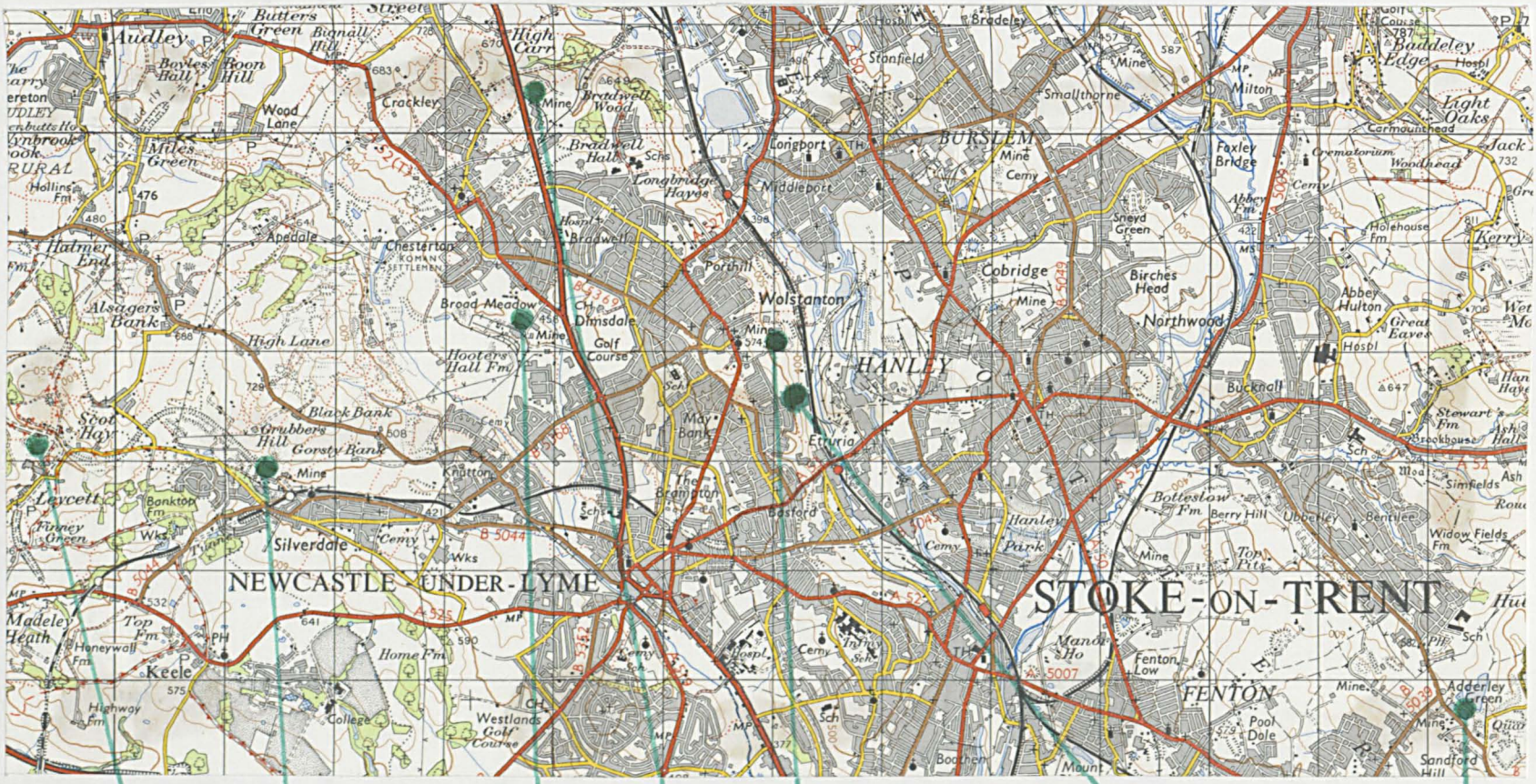
Introduction

The presence in the Stoke-on-Trent area of numerous derelict coal spoil tips offered the opportunity to sample completely artificial habitats which were colonised by higher plants and, therefore, probably by soil micro-organisms. The initial investigations uncovered the presence of numerous unusually warm areas on and around the tips, where internal combustion is responsible for creating these strange micro-habitats. A comparison was drawn between these warm coal spoil tips and a similar situation reported by Loginova et al. (1962). The latter investigated the microflora of the thermal zones of Mount Yangan-Tau (the "burning mountain") in the Southern Urals. The temperature of the gases at the mouths of fissures and artificial boreholes varies from 40-75°C. Their results showed that these thermal zones are extremely rich in thermophilic micro-organisms. They isolated eight fungal cultures capable of developing at 43-49°C, most belonging to the same species, which was present as a snow-white powdery bloom. No further identification has been proposed and

from the temperature relations it would appear to be a thermotolerant species.

The fissures found on warm coal tips had similar temperature relations to those recorded on Mount Yangan-Tau and were descriptively similar. Preliminary analyses of these spoil tips revealed a great variety of thermophilic and thermotolerant species, many of which had not previously been encountered in the earlier studies of natural habitats. It was, therefore, decided to make an extensive survey of these habitats in regard to the thermophilic fungal microflora, especially due to the lack of literature on the subject. It was discovered that a previous worker, Price (1961) had made a detailed study of the fungal microflora of the pit heaps of South Wales. The latter author screened only for mesophiles and hence failed to record any thermotolerant or thermophilic fungi. He did conclude that the fungal microflora of the pit heaps investigated was qualitatively similar to that found in the surrounding areas but was quantitatively very inferior.

MAP TO SHOW THE DISTRIBUTION OF THE MAIN COAL SPOIL TIPS INVESTIGATED



i. Leycett

ii. Silverdale

vi. b) Holditch

vi. a) Parkhouse

iii. Hanley

v. Shelton (Iron & Steel)

iv. Adderly Green

Scale: 1 inch = 1 mile

Spoil tips studied

i. Leycett

Situated about three miles from Newcastle-under-Lyme, this tip was one of the most extensively studied due to its varied and well developed vegetation. The main tip has been derelict for at least twenty years, whilst the smaller subsidiary flat-topped tips have not been disturbed for twenty to thirty years, thus affording a considerable time for vegetation to establish itself and the soil microflora to have reached some level of stability. The main tip is about 300 ft. high and composed of burnt red shale plus coarse grey shale and slag. Wind-borne soil has been established on the north side of the tip supporting *Betula* saplings and bracken. The remainder of the tip is sparsely clothed with *Agrostis* sp., *Senecio jacobea*, *Equisetum* sp., *Chamaenerion angustifolium*, *Cirsium vulgare* and various mosses.

The subsidiary tips have a fairly well developed top-soil above coarse grey shale. This supports numerous *Betula* trees (twenty to thirty years old) and occasional *Salix* species. *Agrostis* sp., *Festuca ovina*, *Bryum*

argenteum and Cladonia sp. are the main colonisers. Lotus corniculatus, Rubus fruticosus, Sonchus sp. and Chamaenerion angustifolium occur sporadically. (Plate 1a)

Sampling dates	pH range	Average pH range
May 1967		
August 1967	5.8-8.1	6.3-6.8
June 1968		

In addition to the main sampling times minor samplings were made, primarily of the warm areas, on several other occasions. Six "warm areas" were sampled on each occasion. Here the vegetation is sparse being chiefly represented by stunted grasses and several moss species, viz: Bryum argenteum and Funaria hygrometrica. Temperatures were found to vary from 26-53°C, the higher temperatures being recorded around the fissures, which emit a mixture of steam and other gases (probably sulphurous in origin). Indeed, in several of the areas no growth is detectable probably due to the presence of extremely high temperatures and poisonous products. Incubation of this spoil material on agar plates often resulted in the production of gases and other by-products

a. Heavily colonised subsidiary tip with part of sparsely colonised main tip in the background.

Plate 1 Leycett coal spoil tip

b. Part of main tip in process of being removed showing the exposure of actively burning areas.

which caused partial dissolution of the lids of plastic petri plates, probably therefore, an inhospitable medium for growth of organisms. In addition, ten "non-warm areas" were sampled, these being composite samples from around the various plant species situated on the main and subsidiary tips.

The wide pH range is probably due to the various forms of mineral deposits present in the slag.

Results and Discussion

"Warm areas" from an analysis of 100 plates.

"Non-warm areas" from an analysis of 120 plates.

The results are presented in Table 14. The total number of isolates recorded from the warm areas was 403 (4.0 isolates/plate) and from the non-warm areas 1120 (9.3 isolates/plate). There were also considerable differences in the number and types of species isolated. Twelve species were isolated from the warm areas and twenty species from the non-warm areas. The dominant species recorded from warm areas were: Rhizopus sp. 1, Aspergillus fumigatus and Allescheria terrestris. Rhizopus sp. 1 was by far the most frequently isolated species encountered, occurring on 43% of the isolation

Table 14

Species present in Leycett spoil material

Species	Warm areas			Non-warm areas		
	no. isol.	% fr. isol.	% occ.	no. isol.	% fr. isol.	% occ.
Absidia ramosa	-	-	-	6	0.5	3.3
Mucor pusillus	43	10.7	12	261	23.3	70.0
Rhizopus sp.1	98	24.3	43	16	1.4	3.3
Allescheria terrestris	69	17.1	21	34	3.0	6.6
A. nidulans	-	-	-	20	1.8	5.0
Chaetomium thermophile var coprophile	-	-	-	25	2.2	6.6
Dactylomyces crustaceus	-	-	-	16	1.4	3.3
Talaromyces duponti	3	0.7	3	4	0.3	1.7
Talaromyces emersonii	2	0.5	1	11	1.0	5.0
Thermoascus aurantiacus	-	-	-	68	6.1	14.2
Trichophaea sp.	37	9.2	15	1	0.1	0.8
Aspergillus fumigatus	75	18.6	24	377	33.7	58.3
A. fumigatus (brown var.)	-	-	-	10	0.9	4.2
A. terreus	-	-	-	11	1.0	5.0
Aspergillus sp. 1	10	2.5	4	-	-	-
Aspergillus sp. 2	5	1.2	3	-	-	-
Chrysosporium sp.	-	-	-	5	0.4	2.5
Humicola insolens	-	-	-	3	0.3	1.7
Penicillium piceum	-	-	-	9	0.8	2.5
Penicillium sp. 1	16	4.0	9	-	-	-
Sporotrichum thermophile	5	1.2	3	6	0.5	2.5
Thermoidium sulphureum	-	-	-	5	0.4	3.3
Thermomyces lanuginosus	40	9.9	12	232	20.7	69.0
Total isolations	403			1120		
Number of species	12			20		

plates. Several interesting species, including Rhizopus sp. 1, Penicillium sp. 1, Aspergillus sp. 1 and 2 and Trichophaea sp., were either unique to the warm areas or found in significantly higher proportions than in the non-warm areas. From this area the three most frequently isolated species were: Mucor pusillus, Aspergillus fumigatus and Thermomyces lanuginosus. It is interesting to note that the other seventeen species were isolated in extremely small numbers, as these three species accounted for 87.7% of the total isolations.

The dominance of certain common species in the non-warm areas reflects, to some extent, the results obtained from the natural areas investigated. The high temperatures encountered in the warm areas, allied with the probable lack of nutrients (see plate 6a) may account for the widely differing results which were found to exist between the warm and non-warm areas. These factors may also encourage species known to colonise burnt ground (pyrophiles) and thus may include species which possess specialised abilities suited to these habitats. For example, Trichophaea abundans (Karst.) Boud., which appears to be closely related to the Trichophaea sp. isolated in the present study, is a known coloniser of

burnt ground and has a fairly wide temperature range, a factor which may assist this species in the colonisation of burnt ground. In the warm areas of coal spoil tips a further factor, i.e. the presence of sulphurous products, may also be responsible for the considerable differences which were found to exist in the thermophilous fungal populations of the warm and non-warm areas of Leycett coal spoil tip.

ii. Silverdale

This is a working colliery situated about half a mile from Leycett. Extensive subsidiary tips form a large complex several hundred yards from the main tip now being used. This complex was originally part of the iron extracting works (now obsolete) and has a characteristic composition of fine and coarse grey shale. The main coal spoil tip is comprised chiefly of red burnt slag resulting from oxidation of the iron pyrites at very high temperatures.

Sampling dates	pH range
May 1967	
April 1968	5.2-7.3
October 1968	

The numerous waste products resulting from the oxidation reactions, together with the effect of vegetation, probably accounts for the diverse range of pH encountered.

The subsidiary tips are composed of high ridges, flattened at the top and supporting a heavy growth of Betula verrucosa (thirty to forty years old) and a ground cover of dense Festuca ovina with patches of

Dactylis glomerata, Agrostis sp. and various mosses and lichens (Cladonia sp.). The sides of these ridge tips are sparsely covered with vegetation, probably due to the lack of stability of the coarse shale and subsequent erosion. An extensive complex of much smaller tips and hollows, with dense vegetation surrounds the large ridge tips. Typical wasteland weeds are abundant in this area and large stands of Salix sp. occur in the sheltered hollows. Recent ground disturbances have created extensive warm areas due to internal combustion of the inflammable spoil tip material. This is noticeable due to the occurrence of fissures which emit steam and other gases (Plate 2a). That sulphur is abundant is proven by noticeable sulphurous odours and patches of yellow sulphur crystals formed around some of the fissures (Plate 2b). A reduced flora of hardy grasses (Agrostis sp.) and moss clumps occurs in these warm areas and often white matted actinomycete growth is present over decaying vegetation. The mosses include Bryum argenteum, Funaria hygrometrica and Leptobryum pyriforme.

A comparison was again made of "warm" and "non-warm" areas. In all examples the temperature of the latter

a. Warm, moss covered area.

Plate 2 Silverdale coal spoil tip

b. Extreme habitat of high temperature and sulphur products.

areas was several degrees below, or similar to, that of the air temperature. The temperatures recorded from the "warm" areas ranged from 32-53°C.

Results and Discussion

"Warm areas" from an analysis of sixty plates.

"Non-warm areas" from an analysis of eighty plates.

The results are presented in Table 15. The total number of isolates recorded from the warm areas was 716 (11.9 isolates/plate) and from the non-warm areas was 668 (8.3 isolates/plate). A relatively wide range of species was isolated - thirteen species from the warm areas and fifteen species from the non-warm areas; nine of these species were common to both areas. The dominant species recorded from the warm areas was Aspergillus fumigatus, with a frequency of isolation of 39.7%. Also well represented was Rhizopus sp. 2, which accounted for 19.4% of the total isolations. The remaining species were isolated in small amounts, although Talaromyces emersonii occurred on over one-third of the isolation plates (36.7% occurrence). A. fumigatus was also the most frequently isolated species from the non-warm areas, comprising 23.8% of the total isolations,

Table 15

Species present in Silverdale spoil material

Species	Warm areas			Non-warm areas		
	no. isol.	% fr. isol.	% occ.	no. isol.	% fr. isol.	% occ.
Absidia corymbifera	2	0.3	1.7	-	-	-
Absidia ramosa	-	-	-	3	0.4	2.5
Mortierella sp.	14	2.0	10.0	-	-	-
Mucor pusillus	29	4.1	16.7	94	14.1	60.0
Mucor sp.	-	-	-	7	1.0	3.7
Rhizopus sp. 2	139	19.4	53.3	-	-	-
Allescheria terrestris	43	6.0	28.3	34	5.1	12.5
A. nidulans	-	-	-	4	0.6	2.5
Chaetomium thermophile var dissitum	4	0.6	3.3	17	2.5	5.0
Dactylomyces crustaceus	2	0.3	1.7	10	1.5	6.2
Talaromyces duponti	-	-	-	8	1.2	5.0
Talaromyces emersonii	70	9.8	36.7	53	7.9	26.2
T. emersonii var. 1	-	-	-	4	0.6	1.2
Thermoascus aurantiacus	46	6.4	16.7	112	16.8	52.5
Aspergillus fumigatus	284	39.7	68.3	159	23.8	63.7
A. fumigatus: orange var.	23	3.2	8.3	11	1.6	5.0
A. fumigatus: olive var.	-	-	-	20	3.0	10.0
Scolecobasidium sp.	9	1.2	5.0	-	-	-
Thermomyces lanuginosus	51	7.1	20.0	132	19.8	60.0
Total isolations	716			668		
Number of species	13			15		

a much reduced level when compared with the previous result from the warm areas. Thermomyces lanuginosus, 19.8% of the total isolations, and Thermoascus aurantiacus, 16.8% of the total isolations, also formed a substantial proportion of the isolates recorded from the non-warm areas.

Two interesting species, isolated exclusively from the warm areas, were recorded for the first time, viz: Mortierella sp. and Scolecobasidium sp. They may be associated specifically with the warm conditions which occur in these habitats. The higher number of isolations recorded from the warm areas compared with that from the non-warm areas may be due to the temperature effect which in the warm areas would be more conducive to the thermophilous growth habit. This must, of course, be linked directly with the amount of available nutrients present and in the warm areas there would appear to be, from the amount of decaying vegetation present (see Plate 2a) which is a result of the rapid plant turn-over, no apparent shortage of nutrients (substrates). The non-warm areas, in general, are not lacking in colonisable substrates and thus, the differences in the number of isolates recorded would appear to be solely

due to the more favourable physical conditions, specifically temperature, present in the warm areas.

However, the obvious presence of sulphur deposits may tend to exert a selective effect on the number (variety) of species isolated and may, therefore, not reflect optimum conditions for the growth of thermophilous fungal species, which might otherwise exist in these warm areas.

iii. Hanley deep

Until recently this pit was functional and the pit buildings still remain intact. It is situated in the centre of the Stoke-on-Trent conurbation and the tips and wasteland cover about one square mile. There are several large tips (200-300 ft. high) and a complex of smaller tips. (Plate 3a)

Sampling dates	pH range
November 1967	
May 1968	6.0-7.3
September 1968	

The vegetation is scarce in comparison with that at Leycett and Silverdale, and comprises chiefly scattered clumps of Agrostis tenuis, Festuca ovina and occasional weeds, e.g. Senecio vulgaris, Sonchus sp. An extensive warm area is present consisting of several parallel fissures which cover sixty to eighty yards, the whole area being bathed in steam and various gases. Temperatures of 24-50°C have been recorded for this area. A distinct ground flora is present which appears to be selected by a temperature gradient.

1) At the lower temperatures (25-30°C) extensive

a. Hanley deep spoil tip with a sparse plant cover.

Plate 3

b. Shelton spoil tip showing the extensive ridge and much denser vegetation.

patches of Chenopodium bonus-henricus occur. This species is noted to grow on warm compost and manure heaps and, therefore, must have some measure of heat tolerance.

2) At slightly increased temperatures low-lying clumps of Agrostis sp. are found, consisting of dead culms and young shoots.

3) At the higher temperatures (35-50°C) a moss species is found present in the spoil material immediately surrounding the fissures. This grows in dense, dark green clumps and is continually bathed in the gases issuing from the fissures.

Results and Discussion

The results are presented from warm and non-warm areas, each area being an analysis of sixty plates, and these are presented in Table 16.

The total number of isolations recorded from the warm areas was 472 (7.9 isolates/plate) and from the non-warm areas was 270 (4.5 isolates/plate). As well as the significant quantitative differences between the two areas, there is also a marked qualitative difference in the variety of species recorded from each area.

Table 16

Species present in Hanley deep spoil material

Species	Warm areas			Non-warm areas		
	no. isol.	% fr. isol.	% occ.	no. isol.	% fr. isol.	% occ.
Absidia corymbifera	5	1.1	5.0	-	-	-
Mucor pusillus	67	14.2	51.7	85	31.5	48.3
Mucor sp.	13	2.7	10.0	4	1.5	3.3
Rhizopus sp. 2	58	12.3	20.0	-	-	-
Allescheria terrestris	16	3.4	8.3	13	4.8	8.3
A. nidulans	-	-	-	4	1.5	3.3
Dactylomyces crustaceus	6	1.3	5.0	-	-	-
Talaromyces emersonii	-	-	-	5	1.8	5.0
Thermoascus aurantiacus	6	1.3	5.0	11	4.1	8.3
Trichophaea sp.	4	0.8	3.3	-	-	-
Aspergillus fumigatus	283	50.4	80.0	141	52.2	85.0
A. fumigatus: orange var.	10	2.1	8.3	-	-	-
Aspergillus sp. 3	3	0.6	1.7	-	-	-
Aspergillus sp. 4	6	1.3	5.0	-	-	-
Scolecobasidium sp.	24	5.1	10.0	-	-	-
Sporotrichum thermophile	-	-	-	7	2.6	6.7
Thermomyces lanuginosus	16	3.4	15.0	-	-	-
Total isolations	472			270		
Number of species	14			8		

Fourteen species were isolated from the warm areas and only eight from the non-warm areas; five species were common to both areas. The latter group includes Aspergillus fumigatus, which was extremely prevalent in both areas, e.g. 50.4% of the total isolations (80% occurrence) in the warm areas, and 52.2% of the total isolations (85% occurrence) in the non-warm areas. Mucor pusillus was also well represented in both areas, especially in the non-warm areas with a frequency of isolation of 31.5%. However, the previously ubiquitous Thermomyces lanuginosus was only occasionally isolated from the warm areas and was completely absent from the non-warm areas investigated. Several interesting species, previously isolated from the warm areas of coal spoil tips, were also recorded from the similar areas on Hanley deep, e.g. Rhizopus sp. 2, Trichophaea sp. and Scolecobasidium sp.

The conspicuous quantitative and qualitative discrepancies between the two areas may be accounted for by the same factors as suggested for the Silverdale results, i.e. temperature and available substrates. The warm areas which were most intensively investigated on Hanley deep spoil tip are very extensive and in

parts, support extremely dense plant cover, especially at the less extreme temperature level. This provides a habitat with a wide temperature range (25-50°C) suitable for the growth of most thermophilous fungi, and, by virtue of the comparatively substantial amount of plant material, may provide an area relatively rich in nutrients. This, conversely, cannot be said of the non-warm areas, which generally support a sparse plant population (see Plate 3a) and may be thought to be lacking in vital nutrients.

iv. Adderley Green

This is a large conical tip (about 300-350 ft. high) which is situated near Longton and is surrounded primarily by open countryside. It has been derelict for a number of years and is substantially covered with vegetation during spring and summer. Plants include Chamaenerion angustifolium (abundant), Senecio jacobea, Cirsium sp., Equisetum sp., Rumex crispus and Dactylis glomerata. Trees and shrubs are absent probably due to the steepness of the tip and the unstable composition, chiefly coarse grey shale.

Sampling dates	pH range
October 1967	
May 1968	6.5-7.2
August 1968	

Near the summit of the tip several warm areas occur and here the spoil material consists of coarse red shale (oxidised) knitted together by dense clumps of moss, mainly Bryum argenteum. No extensive fissures are present and temperatures range from 25-38°C.

Results and Discussion

An analysis of sixty plates from each area was made and the results are presented in Table 17. The total number of isolations recorded from the warm areas was 597 (10.0 isolates/plate) and from the non-warm areas was 814 (13.6 isolates/plate). The number of species isolated from the warm areas was twelve and from the non-warm areas was seventeen, of which nine species were common to both areas. Two of the three species unique to the warm areas of this tip have also previously been recorded exclusively from the warm areas of Silverdale spoil tip, viz: *Rhizopus* sp. 2 and *Scolecobasidium* sp. The third species, *Penicillium* sp. 2, was a new and interesting record. *Rhizopus* sp. 1 and *Trichophaea* sp. were once again isolated in small amounts from both areas and would appear to be an interesting part of the thermophilous microflora of coal spoil tips.

The most frequently isolated species, from both areas, was *Aspergillus fumigatus*, although it was much more abundant in the warm areas (52.8% of the total isolations and a 78.3% occurrence), compared with the non-warm areas (22.6% of the total isolations and a 56.7% occurrence). *Thermomyces lanuginosus* was also substantially represented

Table 17

Species present in Adderley Green spoil material

Species	Warm areas			Non-warm areas		
	no. isol.	% fr. isol.	% occ.	no. isol.	% fr. isol.	% occ.
Absidia ramosa	-	-	-	8	1.0	8.3
Mucor pusillus	10	1.7	8.3	107	13.1	56.7
Mucor sp.	8	1.3	5.0	-	-	-
Rhizopus sp. 1	6	1.0	6.7	3	0.4	3.3
Rhizopus sp. 2	24	4.0	15.0	-	-	-
Rhizopus sp. 3	-	-	-	3	0.4	6.7
Allescheria terrestris	12	2.0	10.0	24	2.9	10.0
Chaetomium thermophile var coprophile	14	2.3	11.7	22	2.7	10.0
Chaetomium thermophile var dissitum	2	0.3	3.3	71	8.7	40.0
Chaetomium thermophile var thermophile	-	-	-	46	5.6	13.3
Talaromyces emersonii	-	-	-	94	11.5	21.7
Thermoascus aurantiacus	-	-	-	61	7.5	25.0
Trichophaea sp.	2	0.3	1.7	4	0.5	1.7
Aspergillus fumigatus	315	52.8	78.3	184	22.6	56.7
Aspergillus sp. 5	3	0.5	1.7	2	0.2	1.7
Chrysosporium sp.	-	-	-	6	0.7	3.3
Humicola grisea var thermoidea	-	-	-	3	0.4	3.3
Penicillium sp. 2	2	0.3	3.3	-	-	-
Scolecobasidium sp.	90	15.1	48.3			
Sporotrichum thermophile	-	-	-	4	0.5	5.0
Thermomyces lanuginosus	109	18.3	50.0	172	21.1	71.7
Total isolations	597			814		
Number of species	12			17		

and had the second highest frequency of isolation in both areas. Mucor pusillus, Talaromyces emersonii and Thermoascus aurantiacus were all frequently isolated from the non-warm areas but were absent or poorly represented in the warm areas. Scolecobasidium sp. was isolated in substantial amounts from the warm areas, comprising 15.1% of the total isolations and with an occurrence of 48.3%.

There would appear to be significant differences between the two sets of results and once again temperature and the organic matter content of the spoil material may be limiting factors. The temperature range is much narrower than has previously been encountered and on this tip the warm areas are not substantially represented, generally, having a limited plant cover consisting of clumps of moss. This may lead to a relative lack of humus, and hence available nutrients, in comparison with the non-warm areas which, because of the dense vegetation, would be expected to be rich in organic matter.

v. Shelton

A derelict tip, originally part of the steel works, which dominates the area between Hanley and Newcastle-under-Lyme, (Plate 3b). The tip is extremely vast, pyramidal in shape, but with an extensive ridge leading to the summit (300-400 ft. high). In its composition this tip differs markedly from the previous coal spoil tips examined. Iron deposits (Magnetite etc.) form the main bulk of the tip together with a variety of waste material. The texture of the "topsoil" is that of a fine black powder and supports a luxuriant plant cover. The latter consists of Lolium perenne, Poa annua, Agrostis stolonifera, Festuca ovina, Artemisia vulgaris, Aster sp., Senecio squalidus and occasional Crataegus monogyna.

Sampling dates	pH range
May 1968 October 1968	5.8-7.5

Along the ridge towards the summit extensive warm areas occur, (Plate 4). At temperatures of 25-40°C vast clumps of Ceratodon purpureus together with Marchantia sp. are abundant, (Plate 5). The black topsoil is particularly dense and steam issues freely when the moss stands are

Plate 4

Shelton spoil tip

Warm area near the summit showing luxuriant growth of moss and higher plants.

removed. A noticeable fact is the absence of sulphurous odours from the vapour and this must be due to the overall lack of coal mining waste products. At the higher temperatures (35-50°C) located around the fissures, a baked thin topsoil occurs supporting only a thin growth of Bryum argenteum.

Results and Discussion

An analysis of fifty plates from each area was made and the results are presented in Table 18.

The total number of isolations recorded from the warm areas was 647 (13.0 isolates/plate) and from the non-warm areas was 429 (8.6 isolates/plate). Also a larger number of species, nine, was isolated from the warm areas as against six from the non-warm areas, of which only three species were common to both areas. Thermomyces lanuginosus (40.3% of the total isolations), Aspergillus fumigatus (33.1%) and Mucor pusillus (15.6%) comprised nearly 90% of the total isolations in the non-warm areas. This shows a close similarity to the results from natural areas, as previously analysed. A. fumigatus also formed a high percentage (48.7%) of the total isolations recorded from the warm areas, but M. pusillus was poorly represented

Table 18

Species present in Shelton spoil material

Species	Warm areas			Non-warm areas		
	no. isol.	% fr. isol.	% occ.	no. isol.	% fr. isol.	% occ.
Mortierella sp.	27	4.2	18	-	-	-
Mucor pusillus	43	6.7	36	67	15.6	62
Rhizopus sp. 2	162	25.0	78	18	4.2	14
Allescheria terrestris	32	4.9	20	-	-	-
Chaetomium thermophile var dissitum	-	-	-	21	4.9	40
Myriococcum albomyces	-	-	-	8	1.9	12
Aspergillus fumigatus	315	48.7	68	142	33.1	80
A. fumigatus: orange var.	11	1.7	6	-	-	-
Scolecobasidium sp.	48	7.4	30	-	-	-
Scopulariopsis sp.	4	0.6	6	-	-	-
Hyphomycete sp.	5	0.8	8	-	-	-
Thermomyces lanuginosus	-	-	-	173	40.3	84
Total isolations	647			429		
Number of species	9			6		

and T. lanuginosus was totally absent from these areas. Rhizopus sp. 2 was frequently recorded from the warm areas, forming 25% of the total isolations and with a 78% occurrence.

Once again, Scolecobasidium sp. comprised a substantial proportion of the warm area isolates and was unique to these areas, as also was Mortierella sp., isolated previously only from the warm areas of Silverdale coal spoil tip. Two Hyphomycetes, not previously recorded, were also isolated in small amounts from the warm areas.

There are significant quantitative differences between the results from the two areas investigated. The results from the non-warm areas reflect, in general, those compiled from natural areas, i.e. a small number of species dominated by several common species. Conditions conducive to a thermophilous growth type are apparent in the warm areas of Shelton spoil tip, and in this instance the high temperatures may be the sole factor involved in determining the higher number of isolations and the wider range of thermophilous fungal species recorded from warm areas, when compared with the non-warm areas. However, the wide temperature range and the presence of luxuriant vegetation (see Plates 4 and 5), suggesting no lack of

a. Moss covered ridge with soil thermometer and *Artemisia vulgaris* in foreground.

Plate 5 Shelton spoil tip

b. Close up to show *Ceratodon purpureus* and *Marchantia* sp. near a small steam-emitting fissure.

organic matter in the surface layers, coupled with the apparent absence of sulphur products, might be thought to encourage a greater variety in the thermophilous mycoflora than was previously recorded from coal spoil tips. Nevertheless, these suppositions are not supported by the results; the total number of isolations are in excess of those previously recorded but there was an overall decrease in the number of species isolated. This may be due, in part, to the abundance of Rhizopus sp. 2 which tended to over-run the soil plates, making colony separation very difficult.

There does, however, appear to be some correlation with the warm areas of the other tips, in the actual species listed, e.g. Mortierella sp. and Scolecobasidium sp. Furthermore, two species not previously recorded were isolated from the warm areas of Shelton spoil tip and would appear to be restricted in their distribution.

vi. Other spoil tips

	Sampling date	pH range
a) Parkhouse, Newcastle-under-Lyme	June 1967	5.9-7.2
b) Holditch, Newcastle-under-Lyme	November 1967	7.5-8.1
c) Sneyd, Burslem, Stoke	March 1968	6.4-6.9
d) Berry Hill, Longton, Stoke	March 1968	6.4-7.9
e) Black Bull, Biddulph, Staffs.	November 1967	6.9-7.8
f) Apedale, Newcastle-under-Lyme	November 1967	6.5-7.8
g) Mossfield, Longton, Stoke	July 1967	6.2-7.1

a) Parkhouse

This pit has very recently closed but no tipping has been carried out for several years. The tip is very tall (about 350 ft. high) and not extensively vegetated. The flora is comprised mainly of wasteland weeds, e.g. Chamaenerion angustifolium, Sonchus sp., Rumex sp., Dactylis glomerata and Agrostis sp. There is a complete absence of shrubs or trees. A few warm areas are evident with temperatures ranging from 25-32°C, but only colonised by scattered patches of Bryum argenteum.

b) Holditch

This pit is still actively worked but all waste material is now compressed underground and hence the tip has not been operated for several years. The conical tip (about 200 ft. high) is sparsely populated by Festuca ovina, Stellaria media and Erysimum cheiranthoides. Warm areas occur near the summit and sulphurous vapours are noticeable. The temperature ranges from 26-37°C and in these areas little plant colonisation is evident, except by Stellaria media.

The remaining tips c) to g) are all fairly recently derelict, except the Black Bull, parts of which are still in use. The vegetation, therefore, is very sparse, due to the recent tipping activities and the flora is represented by common wasteland weeds as listed previously. No areas of unusually high temperatures were detected during sampling and material was collected from around the colonising plant rooting systems.

Results and Discussion

Samples from tips a) and b) were taken from warm and non-warm areas and the results are from an analysis of twenty plates for each area, (see Table 19).

For Parkhouse spoil tip, the total number of isolations recorded from the warm areas was 114 (5.7 isolates/plate) and from the non-warm areas was 212 (10.6 isolates/plate). There was no conspicuous difference in the number of species isolated from each area, being nine from the warm areas and eleven from the non-warm areas, although only five of these were common to both areas.

For Holditch spoil tip, the total number of isolations recorded from the warm areas was 270 (13.5 isolates/plate) and from the non-warm areas was 156 (7.8 isolates/plate). The number of species isolated was small, comprising seven from the warm areas and five from the non-warm areas; the latter species all occurred in the warm areas.

There is a common trend between all the results from the two spoil tips. In all the areas Mucor pusillus, Aspergillus fumigatus and Thermomyces lanuginosus were the most frequently isolated species, especially the latter two species. However, there are also obvious differences between these results. For example, the numbers of species and the total number of isolations recorded show an opposite pattern between the warm and

Table 19

Species present in Parkhouse and Holditch spoil material

Species	Parkhouse						Holditch					
	Warm			Non-warm			Warm			Non-warm		
	isol.	% fr.	% occ.	isol.	% fr.	% occ.	isol.	% fr.	% occ.	isol.	% fr.	% occ.
Mucor miehei	-	-	-	5	2.4	15	-	-	-	-	-	-
Mucor pusillus	12	10.5	20	27	12.7	45	35	13.0	80	43	27.5	55
Mucor sp.	3	2.6	10	10	4.7	40	-	-	-	-	-	-
Rhizopus sp. 1	-	-	-	-	-	-	19	7.0	45	12	7.7	25
Rhizopus sp. 2	7	6.1	15	-	-	-	-	-	-	-	-	-
Allescheria terrestris	-	-	-	5	2.4	20	12	4.4	20	-	-	-
Chaetomium thermophile var. coprophile	-	-	-	8	3.8	20	-	-	-	-	-	-
Chaetomium thermophile var. dissitum	7	6.1	15	-	-	-	21	7.8	60	-	-	-
Dactylomyces crustaceus	8	7.0	15	5	2.4	15	-	-	-	-	-	-
Thermoascus aurantiacus	-	-	-	6	2.8	20	3	1.1	10	2	1.3	10
Aspergillus fumigatus	23	20.2	45	65	30.6	80	97	35.9	85	38	24.4	45
A. fumigatus: orange var	5	4.4	10	-	-	-	-	-	-	-	-	-
Humicola insolens	7	6.1	10	-	-	-	-	-	-	-	-	-
Sporotrichum thermophile	-	-	-	6	2.8	15	-	-	-	-	-	-
Tritirachium sp.	-	-	-	4	1.9	10	-	-	-	-	-	-
Thermomyces lanuginosus	42	36.8	65	71	33.5	85	83	30.7	95	61	39.1	60
Total isolations	114			212			270			156		
Number of species	9			11			7			5		

non-warm areas of the two tips. The number of isolations from the warm areas of Parkhouse spoil tip was almost half of that from the non-warm areas, whilst for Holditch spoil tip the exact reverse was true.

An explanation of this anomaly may be solely due to the amount of organic matter present in each area, possibly linked with a temperature effect. The warm areas on Parkhouse spoil tip are relatively scarce and limited in the amount of vegetation present. The more substantial vegetation occurs on the non-warm areas and in this spoil tip the balance which determines the numbers of thermophilous fungal species and isolates would appear to swing in favour of the amount of vegetation (organic matter) present in that area, and not the actual soil temperature, although, in this case, there is a limited temperature range of 25-32°C. There may, therefore, be a nutrient limiting factor in the warm areas which would restrict the numbers of isolations and species recorded.

The warm areas on Holditch are, generally, more extensive than their counterparts on Parkhouse spoil tip, and also have a slightly greater temperature range (26-37°C). However, the vegetation over the whole tip

is comparatively sparse, especially in the warm areas where the plants have a very short life span, possibly due to the adverse conditions. There would, therefore, appear to be a restricted organic matter content which is at a fairly constant level over the whole tip. The higher temperatures which occur in the warm areas may, in this case, alter the balance leading to a greater number of thermophilous species and isolates in these areas when compared with the non-warm areas.

An interesting species, Tritirachium sp., was isolated from Parkhouse spoil tip, and occurred only in small amounts. Rhizopus sp. 1 was again isolated from coal spoil tip material, occurring in the warm and non-warm areas of Holditch spoil tip.

The material from tips c) to g) was not accurately weighed before plating out and so individual colonies were not tabulated. Instead the average frequency of occurrence of each species was calculated. For each sample (four plates) the relative frequency of occurrence of the respective fungal species was estimated, viz:

+ - single colony

1 - present on one plate several times

2 - present on two plates

3 - present on three plates

4 - present on three plates abundantly

5 - present on all plates abundantly

The average for ten samples was then calculated together with the percentage occurrence of each species on forty plates. The results are presented in Table 20.

Comparing these results, Mucor pusillus and Thermomyces lanuginosus were the only species common to all five spoil tips. The relatively frequency of isolation and the occurrence of these species were very high in all cases. Comparatively few species were recorded, the lowest being five and the highest nine, and none of the unusual species, which would appear to be indicative of coal spoil material, were isolated. The only species which were widespread and occurred on three or more of these coal spoil tips were Aspergillus fumigatus, Talaromyces emersonii and Thermoascus aurantiacus. The former species, when present, had a high relative frequency and occurrence. These results show similarities with the results of samples taken from natural soils. In general, a small number of common species were isolated

in large amounts and on a substantial proportion of the plates, whilst the remaining species were recorded in relatively small amounts and had a limited occurrence.

Table 20

Species present in five spoil tips

Species	Sneyd		Berry Hill		Black Bull		Apedale		Mossfield	
	freq.	% occ.	freq.	% occ.	freq.	% occ.	freq.	% occ.	freq.	% occ.
Absidia ramosa	+	2.5	-	-	-	-	-	-	-	-
Mucor pusillus	5	100	4	77.5	5	95	4	87.5	4	80
Mucor sp.	2	25	-	-	2	22.5	-	-	-	-
Allescheria terrestris	2	32.5	-	-	-	-	2	15	-	-
Chaetomium thermophile var. coprophile	-	-	-	-	1	15	-	-	-	-
Chaetomium thermophile var. dissitum	-	-	2	47.5	-	-	-	-	-	-
Dactylomyces crustaceus	-	-	-	-	1	5	1	10	-	-
Talaromyces emersonii	-	-	2	32.5	-	-	1	10	2	37.5
Thermoascus aurantiacus	-	-	3	55	2	25	2	22.5	2	20
Aspergillus fumigatus	5	92.5	-	-	5	100	3	55	5	92.5
A. fumigatus: orange var	-	-	-	-	1	12.5	-	-	-	-
Sporotrichum thermophile	1	10	-	-	+	2.5	-	-	-	-
Thermomyces lanuginosus	4	82.5	5	95	5	80	5	85	4	72.5
Number of species	7		5		9		7		5	

Discussion of coal spoil tip results

Due to the considerable proportion of the sampling devoted to these unusual habitats, in relation to the other areas investigated, a separate discussion is thought to be necessary.

An analysis of the results in order to compare the numbers of isolations and species recorded from all the warm and non-warm areas shows similarities in respect of these criteria. The average number of isolates, per gram dry weight of coal spoil material, from the warm areas was 18.9 and the average numbers of species recorded was 11.0, whilst from the non-warm areas the average number of isolates was 17.9 per gram and the average number of species was 11.7. Hence, there are no marked quantitative differences when the complete results are taken into account.

However, there are specific differences on individual coal spoil tips and, in particular, from the qualitative results, i.e. the types of species isolated. Certain species occurred exclusively from the warm areas of several tips, e.g. Mortierella sp. and Scolecobasidium sp., whilst other species have been recorded in substantially

higher numbers in the warm areas of a number of tips, when compared with their frequency in the non-warm areas, e.g. Trichophaea sp., Rhizopus sp. 1 and Rhizopus sp. 2. All these species seem to show special affinities for these warm areas and the majority of them have a distribution unique to coal spoil tips in particular. In fact, Rhizopus sp. 1 was isolated from rabbit pellets (see Table 9) but these were collected on a coal spoil tip. Furthermore, a number of other interesting species, not previously encountered, have been isolated from coal spoil tips, e.g. Penicillium spp. 1 and 2, Tritirachium sp., Aspergillus species and two un-named Hyphomycetes. Several of these species have been isolated exclusively from the warm areas of the tips, but never on more than one tip. They would appear, therefore, to have a limited distribution and occurrence and may be favoured by the unusual environment, especially in the warm areas, which exists on the coal spoil tips.

It must be taken into consideration that the coal spoil tips were sampled and studied in much greater detail than the natural and semi-natural areas investigated. The sheer weight of number of samples analysed would, of course, enrich the number of species recorded when compared with

the relatively small proportion of samples taken from the other areas. Nevertheless, the considerably larger number of species isolated from coal spoil tips, and the apparent absence of certain species from natural and semi-natural areas, would suggest the suitability of the former habitats as rich sources of thermophilous fungi. The temperature effect in the warm areas may play a large part in selecting those species best suited to the conditions in a habitat which will restrict competition from mesophiles. Due to ground disturbances any part of the tip may be subject to internal combustion and an area may turn, practically over-night, from one of normal temperatures to one of markedly elevated temperatures (see plate 2b), with a consequent change in the vegetation pattern. The stability of these warm areas is, therefore, difficult to assess and so it is impossible to estimate the length of time that they have been present (i.e. active). The period of activity, and specifically the period when the warm areas have been suitable for colonisation by higher plants and fungi, may be a vital factor in determining the numbers of species and isolates which occur in the warm areas of any one particular spoil tip. Those areas, which now have normal vegetation and surface temperatures

may have been at one time, subject to high surface temperatures and this may explain the occurrence of several of the unusual species in these non-warm areas, although spore dispersal may, of course, account for their occurrence.

There are gradual phases leading to the establishment of a flora in the warm areas. Firstly, colonisation is by hardy mosses, followed by similarly hardy grasses. In these conditions there is a rapid plant turn-over due to the extremes of heat and lack of a suitable "soil" crumb. Ultimately, with the cooling down of these areas and the presence of air-borne soil, common wasteland weeds and shrubs begin to dominate. With the gradual build-up of topsoil, humus, etc., competition in the microflora will greatly increase. The lower the temperature, the more the conditions will benefit the majority of mesophiles and only the thermotolerant species of the thermophilous mycoflora will be able to compete. The survival of the obligate thermophiles will depend on the production of numerous spores (asexual or vegetative) or overwintering structures (sexual or vegetative). These species will then be subject to the same growth patterns that exist in normal soils (i.e. Section I) e.g. short growth cycles during periods of high local temperature caused by solar

insolation or rapid microbial activity.

The apparent high sulphur content of the spoil material, especially noticeable from the gases present in the warm areas, is another factor which may affect fungal distribution and lead to the exclusion of certain species. Generally, the humus and therefore the nutrient content of coal spoil tips is low and little or no soil structure is present and, except in the tips with substantial vegetation, they remain inhospitable environments. The competition for available nutrients will probably be intense, but in the warm areas competition will be reduced by the selective effect of temperature, and in these regions the thermophilous fungi, better adapted to the conditions, will form the bulk of the fungal population. Cornwell and Stone (1968) have investigated the availability of nitrogen to plants in acid coal spoil tips. They showed that the nitrogen available may be very abundant in certain spoil material especially the strongly acidic shales. However, the less acidic shales - i.e. pH 4.6-5.2 - may have a nitrogen deficiency due to a minimal rate of sulphide oxidation and silicate destruction which normally would liberate "fixed ammonium" as well as other nitrogen-containing compounds. The great majority of the

coal spoil tips investigated during the present study were weakly acidic or neutral and hence in these spoil tips nitrogen deficiency may occur and so lead to a reduced flora and mycoflora.

Plant colonisation will enhance the organic matter content of the spoil material and this will be a most important factor in determining the fungal populations occurring in the warm and non-warm areas. In particular, the thermophilous mycoflora will also be substantially influenced by the temperature of the spoil material. There would appear therefore, to be a balance between the availability of nutrients and the availability of sufficiently high temperatures to promote the thermophilous type of growth. Those spoil tips in which the warm areas are sparsely colonised by higher plants and generally lacking in humus (see Plate 6a) and a specific soil structure, e.g. Leycett, Adderley Green, Parkhouse, consistently recorded a much lower number of isolations and species when compared with the non-warm areas of those tips. The latter areas usually show some soil structure and varying plant colonisation. Furthermore, the number of isolates from the non-warm areas of spoil tips with substantial vegetation was fairly constant,

a. Poor colonisation at Leycett coal spoil tip.

Plate 6

b. Dense colonisation at Shelton iron spoil tip.
Close up of Ceratodon purpureus and Aster sp.

averaging about 11.0 per plate. However, the number of isolations from the non-warm areas of Hanley deep spoil tip, which has a sparse plant cover, was notably small, i.e. 4.5 isolates per plate and this must reflect the shortage of nutrients. In contrast, nearly twice as many isolates and also nearly double the number of species were recorded from the warm areas of this tip, where increased temperatures and a denser plant population would favour the growth of thermophilous fungi.

Those tips which have well colonised warm areas, and hence a high organic matter content, e.g. Silverdale, Shelton (see Plate 6b), consistently showed higher isolate counts in comparison with the non-warm areas of the same tip, which are also substantially colonised.

e.g.

Silverdale	Number of isolates/plate	Number of species recorded
Warm area	12.0	13
Non-warm area	8.3	15
Shelton		
Warm area	13.0	9
Non-warm area	8.6	6

There would, therefore, from the above results, appear

to be a significant difference between the warm and non-warm areas, in respect of the number of isolations recorded, when the organic matter content is not a limiting factor. However, the number of species isolated was not significantly different between the warm and non-warm areas. Nevertheless, the actual species composition of the warm areas is interesting, in particular the Mortierella sp. and Scolecobasidium sp., which were not located in the non-warm areas of these tips. Also other species have been recorded from non-warm areas but not from the warm areas, e.g. Myriococcum albomyces and Thermoidium sulphureum, and these may be unable to adapt to the conditions in the latter areas.

The ultimate source of some of the unusual species recorded, and especially those which appear to have a distribution associated with coal spoil tips, is puzzling. The species must be transported to the tips during initial colonisation by air currents etc., and the large conical tips such as Adderley Green and Parkhouse, which dominate the landscape for miles around, will act as giant spore traps and thus attract a wide range of fungal species. The warm areas will in this way be colonised by the most suitably adapted species, i.e. thermophilous fungi, which

will tend to flourish in these habitats provided suitable nutrients are present. The older tips, such as Leycett and Adderley Green are relatively rich in thermophilous fungal species, compared with the newer tips, viz: Hanley deep, Holditch. Therefore, the length of time to which a tip is open to colonisation (i.e. when tipping has stopped) may be important in determining the nature of the thermophilous mycoflora. Adaptation may be an important process during the colonisation of coal spoil tips, specifically in the warm areas. This will involve adaptation to the high temperatures encountered and, perhaps, also to the presence of sulphur products, and may explain the substantial number of interesting species recorded from these areas. It may involve the gradual tolerance (mutation followed by selection) towards heat of an individual species (strain) and over a long period of time may lead to a dependence upon high temperatures for the initiation of growth.

Brock (1967) has investigated the adaptation of blue-green algae to hot springs. In these hot springs considerable temperature gradients occur and he concluded that the algal population at any specific part on the gradient has evolved so that its optimum temperature for photosynthesis is the same as the environmental temperature. This was

true even at temperatures of 70°C, close to the upper limit for growth. Of course, the period of time over which adaptation has taken place is immense but the same fundamental principles may apply to the adaptation of fungal species to similar warm environments.

General discussion of the sampling results

The numbers of isolates per gram dry weight of sampled material and the number of species present, for a specific area, are summarised as follows:

	Area Sampled	Isolates/gm.	Number of species/area
1.	Grassland	18	12
2.	Sand-dunes	12.5	11
3.	Marshland	22.5	8
4.	Pine-woods	15	13
5.	Mixed deciduous woods	22	7
6.	General woodland	15	11
7.	Mountainous areas	7.5	9
8.	Animal droppings	115	9
9.	Manures and composts	178	15
10.	Sewage	66	17
11.	Birds' nests	30	15
12.	Cooling towers	30	11
13.	Coal spoil tips	18.5	42

The results are presented in histogram form in Figures 1 and 2. The sampled areas have also been divided up into their original section groupings, i.e. I. Natural Areas, II. Semi-natural Areas and III. Industrial Areas.

The last two sections comprise those areas where warm conditions are known to occur as part of the 'normal' environment. From an analysis of the results in Figures 1 and 2, two broad observations can be made, viz:

a) the number of isolates, per gram dry weight, is consistently higher for section II and marginally for section III.

b) the number of species recorded is also, in general, consistently higher for sections II and III.

As might be expected, the number of isolates, per gram dry weight, is much higher in those areas where warm conditions prevail, especially marked in manures and composts, in comparison with the normal soils investigated. The relatively low numbers isolated from coal spoil tips, similar to those recorded from some of the natural areas, have previously been explained and it was concluded that the overall lack of organic matter in spoil material may be contributory to the low counts recorded. Although there are conspicuous differences between the composts when compared with those from normal soils, they are numerically inferior to those previously reported by workers investigating similar habitats. Waksman et al. (1939a)

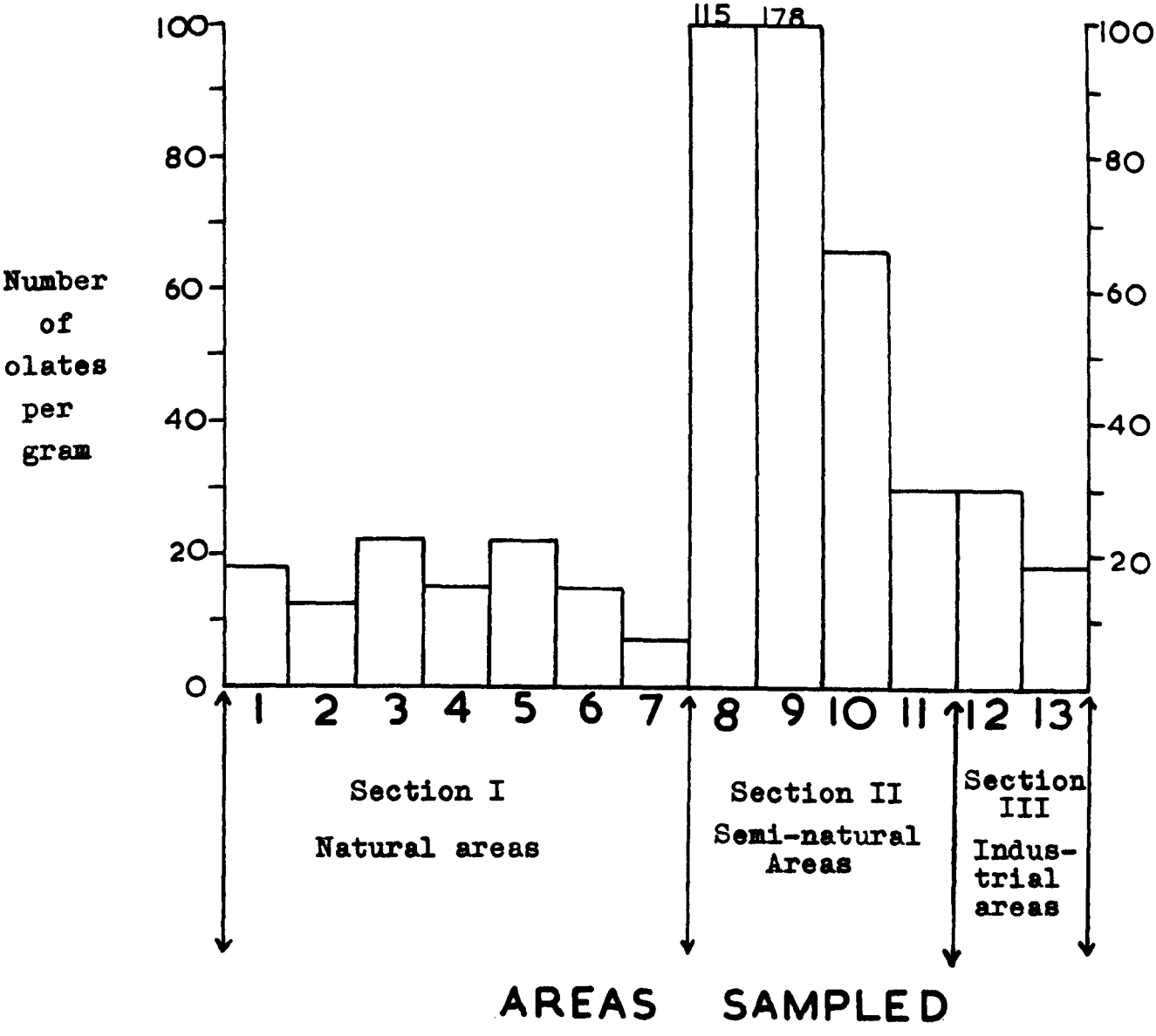


Figure 1

The number of isolates per gram for each area sampled

reported that thermophilous fungi represent 200,000 to 600,000 propagules per gram of compost at 28°C, and at 50°C this figure may reach two million. However, at 65°C no fungi were recorded. Klopotek (1962) recorded the number of fungi isolated from raw refuse at 48°C, the numbers in one gram varied from one hundred to eight thousand and represented 0.173% of the total mesophilic population. In the finished compost or refuse the numbers of fungi isolated at 48°C reach 296,000 per gram. Similarly, high results have been recorded from composts by other workers (Chang and Hudson 1967).

The relatively low numbers of isolates recorded in the present study is difficult to explain. Certain of the manures and composts investigated were in active stages of decomposition and it is during these stages that high counts of thermophilous fungi have been recorded. Nevertheless it is also true that during the middle stages of composting activity the numbers decrease in comparison with those reached during the final stages. The temperature of isolation employed in the present study was similar to that used by previous workers so this cannot account for the discrepancy of results. One factor remains, that is that the actual isolation processes may

bias the results.

During the present study the soil plate method was the only isolation technique used for quantitative results, whereas the workers mentioned previously used dilution plate techniques. The soil plates were usually examined after twenty-four hours and after this period the plates from manures etc., in general became overgrown and individual colony counts were difficult to record. Therefore, the full spectrum of isolates may not have been obtained from the sample being analysed. It is well known that the soil plate method favours fast-growing species but this is also true for the dilution plate technique. The latter method would also tend to favour the occurrence of high-sporing species and they would be recorded in much greater amounts than on soil isolation plates. The argument is that individual colonies on dilution plates arise mainly from single spores due to the extensive mixing involved and the final homogenous nature of the inoculum used. Therefore, vast numbers of colonies may be recorded from a single sporing head located in the original material. However, the soil plate inoculum is much more heterogeneous and a single colony recorded on these plates may have arisen from one 'original'

soil colony, i.e. from a number of sporing heads, due to the fact that a high density of spores may occur on a single soil particle or group of particles and on incubation will be viewed as a single colony. Thus, the numbers of colonies isolated by the two methods may differ considerably, although the actual qualitative results may be similar.

Other workers have estimated the amounts of thermophilous fungi found in natural areas (normal soils). Timonim (1935) recorded the numbers of fungal isolates from virgin soils in Manitoba. In woodland soil the numbers in the surface layers at 37°C ranged from 0-450 per gram dry weight. At lower depths the numbers decreased markedly. Waksman et al. (1939b) in a study of cultivated soils reported that thermophilous fungi were very sparsely represented, in comparison with other soil micro-organisms, with no more than 100 per gram of dry soil. Apinis (1960, 1963a) using dilution plates, indicated that thermophilic fungi in certain alluvial soils near Nottingham never exceeded 200 per gram in the upper layers of these soils, and there were usually fewer than 100 in one gram of dry soil. Apinis (1963b) investigated the thermophilous fungi of coastal grasslands and compared the numbers of these

fungi isolated at 38°C with the fungi (mesophiles) isolated at 20°C. The number of isolates recorded at 38°C was a fraction (usually less than 0.1%) of that recorded at 20°C. The author concluded that the most suitable natural environments of thermophilous fungi are the dead leaves, litter layer and the humus horizon of the grassland. The number recorded from sand (supporting Spartina townsendii) was forty per gram in the surface soil and fifty per gram at a depth of 10-15 cms. These results can be favourably compared with the results of the present investigation of sand-dunes and salt-marsh soil taking into account the much higher temperature of isolation (48°C) employed.

Less accurate quantitative results have been reported by a number of workers investigating the thermophilous fungal populations of normal soils. Craveri et al.(1964) recorded only fifty isolates from twenty-four samples of cultivated soil collected in Northern Italy. The isolation temperature was 50°C but no accurate quantitative data was presented. Stokes and Redmond (1966) failed to isolate any thermophilous fungi from an investigation of uncultivated, cultivated and garden soils and also from lake mud. However, the incubation temperature employed

(55°C) is very high for the majority of thermophilous fungi, although several species grow quite well at this temperature. They estimated the proportion of thermophilic bacteria present in these soils, and found that it represented less than 0.1-1.6% of the total microbial population, and there may be a similar relationship between the thermophilic and mesophilic fungal populations of normal soils.

It can be concluded, therefore, that the thermophilous fungi are not well represented in normal soil and the present results would tend to confirm this conclusion. During this study the number of thermophilous fungi was found to range from eight to twenty-two per gram dry weight of soil, at incubation temperatures of 48-50°C. The incubation temperature of the soil or dilution plates will, of course, have a profound effect on the numbers of fungi recorded and the temperatures used in the present study will tend to reduce markedly the number of isolations, in comparison with the numbers isolated by previous workers using, in general, lower incubation temperatures, i.e. 37-38°C.

Thermophilous fungi would appear to be an integral part of the surface soils of all the natural areas

investigated, although they constitute a minute proportion of the total fungal population found in these areas (probably less than 0.1%). The numbers of these fungi were found to increase dramatically in herbivore dung and manures and composts. They also increased in the other semi-natural areas studied, such as in sewage, where the numbers were three to four times as great, and in birds' nests approximately twice the amount of isolates were recorded, when compared with the soil average. In the cooling towers of the power station an increased thermophilous population occurred, in comparison with normal soils, whilst that of the coal spoil tips would appear to be numerically similar to the latter.

The qualitative aspects, however, would appear to be of a more complex nature. In general, the numbers of species recorded from semi-natural and industrial areas (warm conditions) are in excess of those recorded from normal soils (natural areas), see Figure 2. It must be stressed, however, that the natural areas were investigated only superficially, in order to gain a general picture of the occurrence and distribution of thermophilous fungi in normal soils and to compare those criteria with those existing in known warm areas, and in particular, with coal

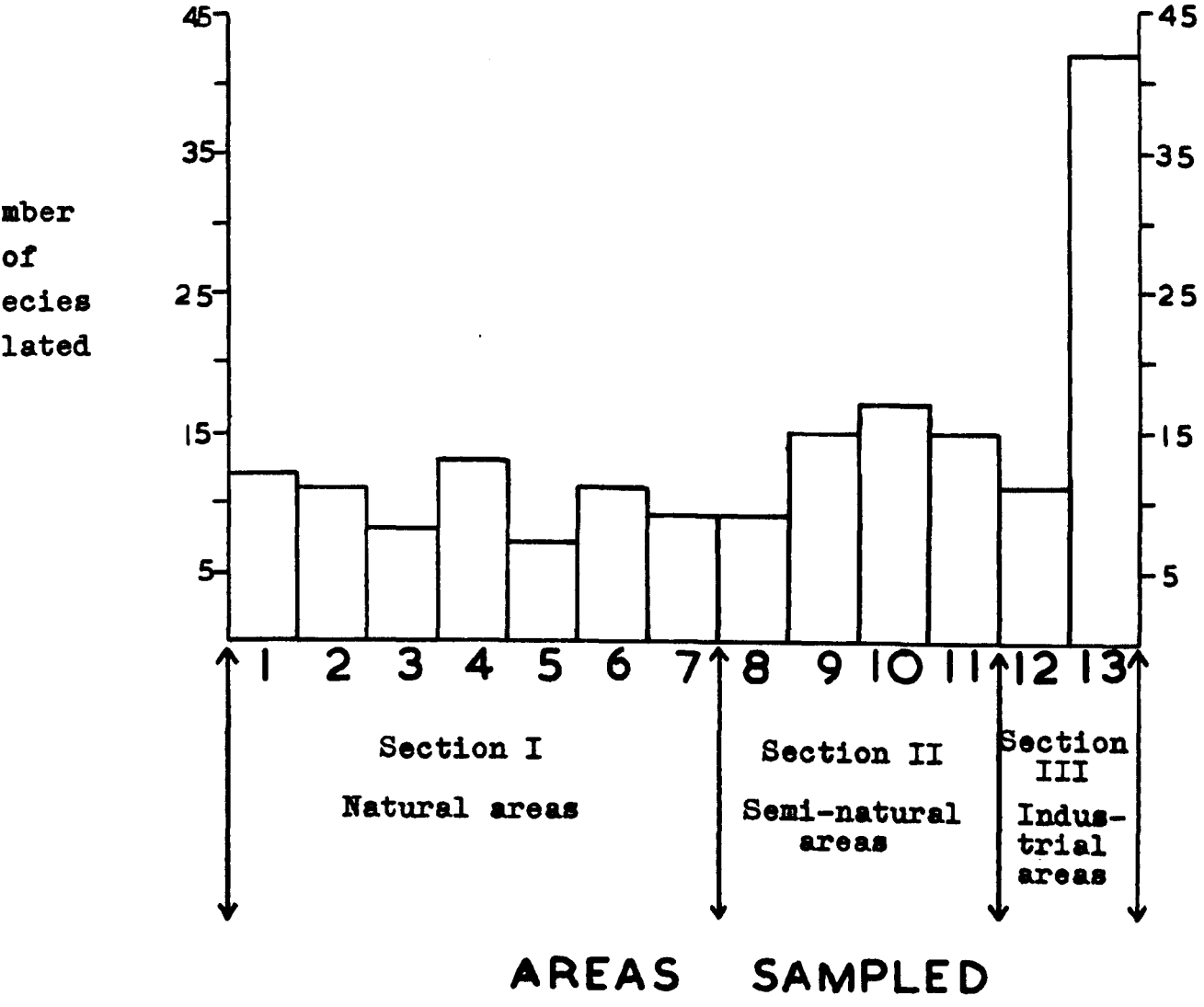


Figure 2

The number of species isolated from each area sampled

spoil tips. It cannot be over-emphasized that the shortcomings of the sampling methods are all too many. For example, the seasonal factors involved in the occurrence of these fungi was not taken into account and also profile studies were not pursued, the soil being taken mainly from the surface layers. However, the initial aim was not to make a detailed analysis of these areas but to make a general survey of the thermophilous species present in the soil at the time of sampling and their relative densities. Several workers have made detailed studies of the thermophilous fungal populations of similar habitats and their results will be discussed later.

Therefore, as might be predicted, the variety of thermophilous species isolated from warm areas (see Figure 2), in comparison with natural areas, was generally greater. However, certain anomalies do exist, namely the relatively few species recorded from herbivore dung. This may suggest that few true coprophiles are represented in the thermophilous mycoflora, but may also suggest the limited period of exposure to fungal colonisation before being sampled. The comparatively small number of species isolated from cooling towers can perhaps be justified by the relative inaccessibility of this habitat to colonisation

by air-borne spores. However, once a species is established in this habitat the conditions will tend to favour the growth of thermophilous fungi, i.e. high temperature and humidity, and this may account for the fairly high isolate counts comparable with those from birds' nests.

From Figure 2, the tremendous rise in the number of species isolated from coal spoil tips is obvious, more than double that recorded from any other single area. This, of course, may be due solely to the larger number of samples taken from the coal spoil tips but it is thought, in part, that this is due to the unusual nature of this habitat which selects out the species most suited to development in this environment. Consistently high temperatures, the overall lack of nutrients, the presence of sulphur products, may all be factors which play a part in eliminating certain species whilst encouraging other species which may otherwise be obscured.

Apinis (1963b) made a detailed study of the thermophilous fungi in coastal grasslands. Of particular interest to the present study was the investigation of sand-dunes and salt-marsh areas. Absidia ramosa, Allescheria terrestris, Aspergillus fumigatus, Chaetomium thermophile, Sporotrichum thermophile, Talaromyces duponti, Thermoidium sulphureum

and Thermomyces lanuginosus were all recorded from these areas. This species list is very similar to the one obtained from similar areas during the present study, (see Tables 2 and 3). Furthermore, several species were isolated which were not recorded by Apinis, e.g. Chrysosporium sp., Myriococcum albomyces and Thermoascus aurantiacus. Allescheria terrestris was not isolated during the present study of these areas and Apinis considered it to be of rare occurrence.

The other habitats studied are less well documented and few comparisons, therefore, can be drawn with previous work. However, there were interesting fluctuations in the types of species recorded, and these were discussed for each individual area.

Nevertheless, one can make several broad groupings of the thermophilous fungi in the range of habitats investigated, viz:

1) Those species which occurred in the majority, if not all, of the habitats, and were usually isolated in substantial amounts.
2) Those species which occurred in a wide variety of habitats, but were generally present in small amounts.

3) Those species which occurred in a limited number of habitats, usually in warm conditions, and may or may not be present in substantial amounts.

4) Those species which only occurred in a single habitat, coal spoil tips in particular, but which may represent an integral part of the thermophilous mycoflora of that area.

Group 1. fungi include: Aspergillus fumigatus, Mucor pusillus and Thermomyces lanuginosus.

The percentage frequency of isolation and occurrence of those species in each habitat are presented in histogram form in Figures 3 to 5.

These three species comprise a substantial part of the total isolations for all the habitats studied. Their combined frequencies of isolation give an average of approximately 72% (i.e. approximately 72% of the total isolations from each habitat). In general, the warmer habitats were dominated less by these three species, although Thermomyces lanuginosus continued, and possibly increased, to form a high proportion of the total isolations (see Figure 5). A. fumigatus and T. lanuginosus in particular were the dominant species in a large proportion of the habitats and the percentage occurrence

of each species was consistently high (see Figures 3 and 5). M. pusillus, in comparison, was less frequently isolated but generally had a high percentage occurrence in each habitat, (see Figure 4).

These three species were also found by Apinis (1963b) to be extremely common in coastal grasslands and were reported as being early colonisers of senescent grass leaves and culms. He concluded that "their wide range may be due to their indifference to various grass substrates and to the microclimatic conditions of a grassland vegetation". From the results of the present study this conclusion can be expanded to include a wide variety of substrates and microclimates. The high frequency of occurrence of A. fumigatus and M. pusillus has also been reported by Küster and Locci (1964) from peat and bogland. Previously, Jensen (1931) reported A. fumigatus to be the dominant form in greenhouse soils representing 35-70% of the total isolations. Also, Pugh (1962) recorded A. fumigatus as being abundant in salt-marsh soils, and it would appear, therefore, that this species, because of its common appearance on isolation plates incubated at lower temperatures, can compete successfully with mesophilic species.

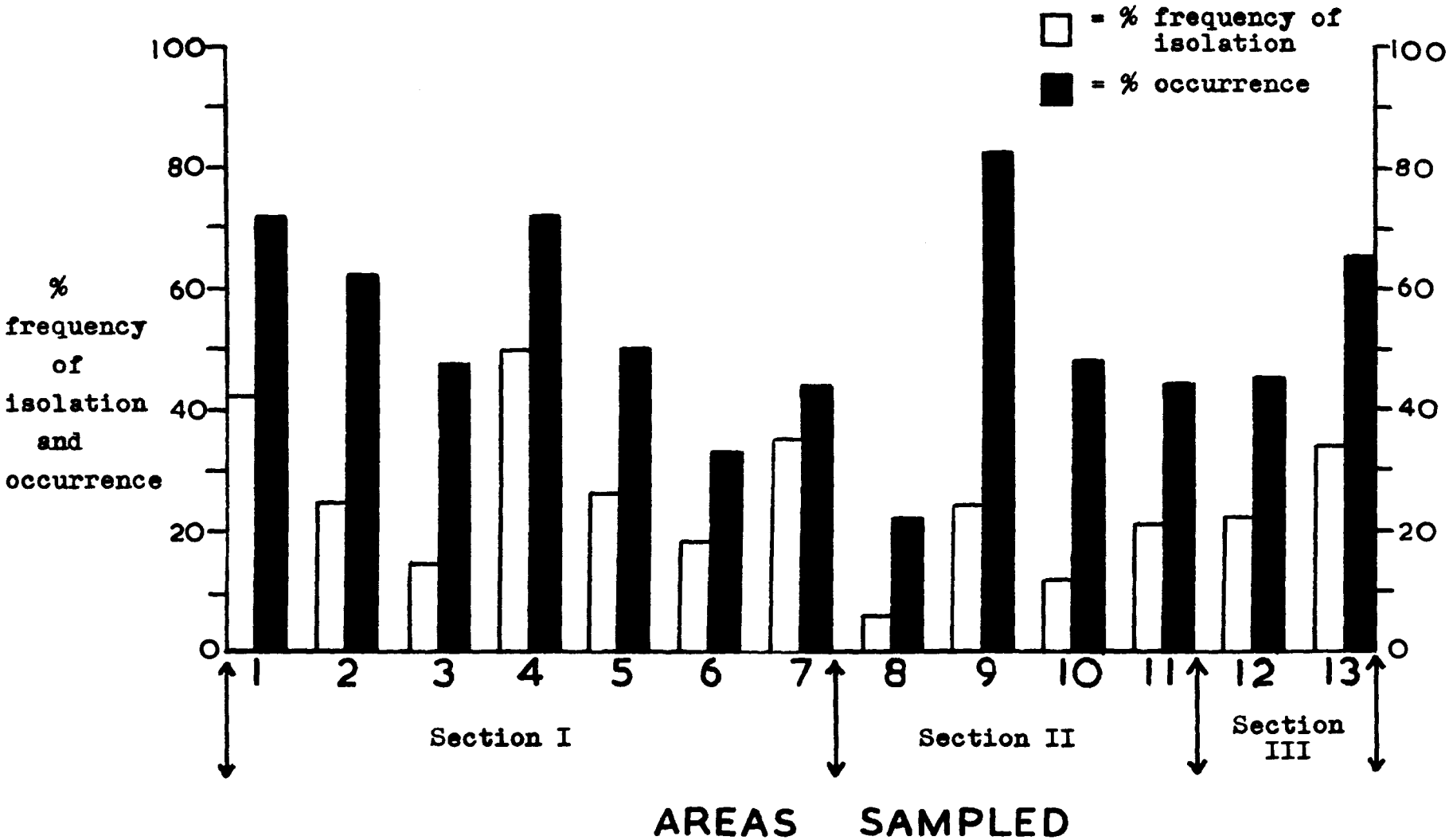


Figure 3

Percentage Frequency of Isolation and Occurrence of Aspergillus fumigatus

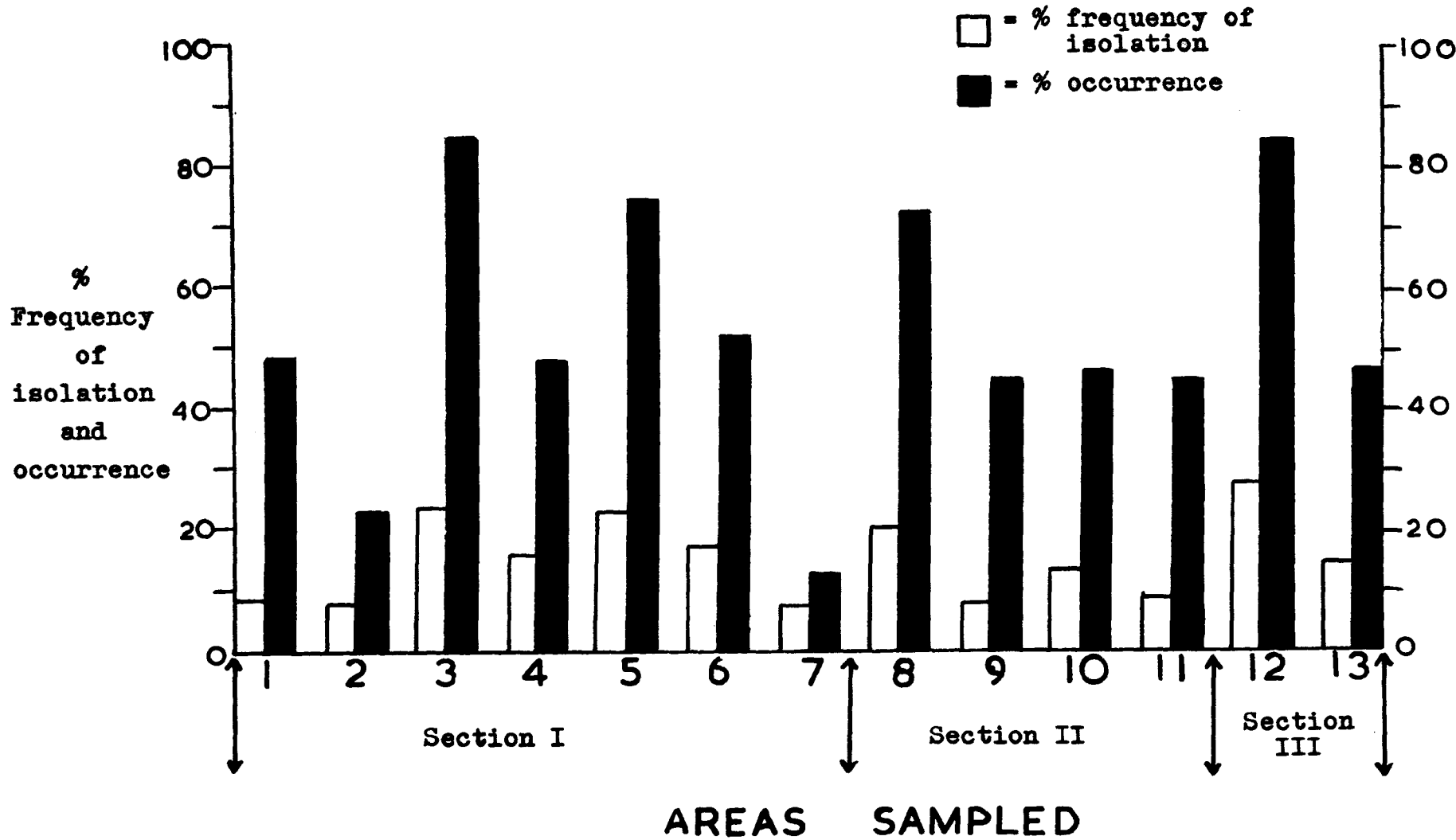


Figure 4

Percentage Frequency of Isolation and Occurrence of Mucor pusillus

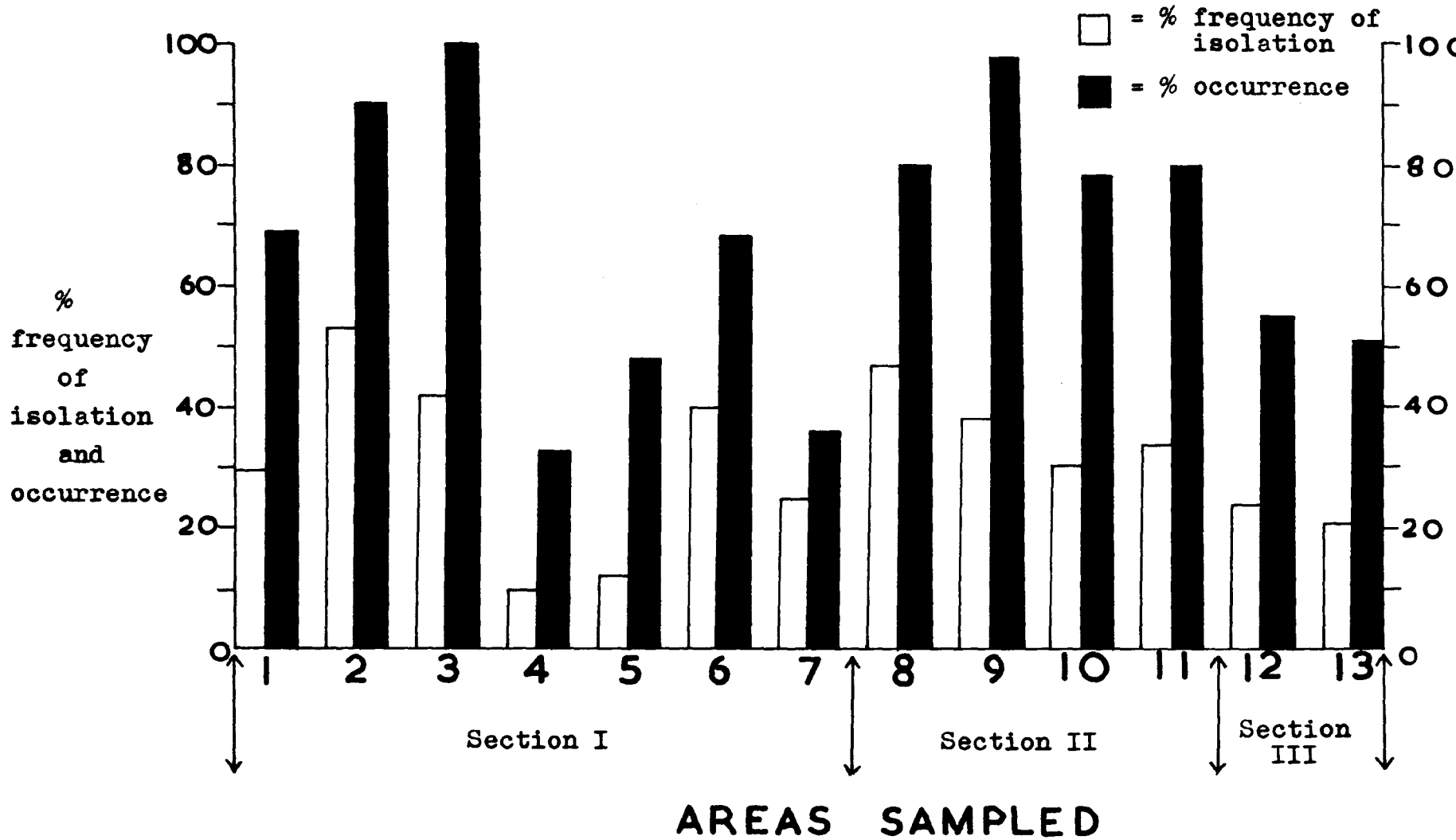


Figure 5

Percentage Frequency of Isolation and Occurrence of Thermomyces lanuginosus

The high occurrence of these three species in various composts and manures, as previously reported (Klopotek 1962, Chang and Hudson 1967), would confirm the ubiquitous distribution of these species in a wide variety of habitats. Nevertheless, Eastwood (1952) during a study of the fungi of straw compost isolated only A. fumigatus as representative of the thermophilous mycoflora.

Group 2 species include: Absidia ramosa, Allescheria terrestris, Chaetomium thermophile, Chrysosporium sp., Dactylomyces crustaceus, Mucor sp., Myriococcum albomyces, Sporotrichum thermophile, Talaromyces duponti, Talaromyces emersonii, Thermoascus aurantiacus and Thermoidium sulphureum.

There are, of course, no fixed boundaries between these artificial groupings and several species are borderline cases. For example, T. duponti and Th. sulphureum could almost be placed in Group 3, but have a slightly wider distribution than the species classified in that group.

Mucor sp., Chaetomium thermophile and Thermoascus aurantiacus occurred in a wide variety of habitats (over 70% of the habitats studied), and the latter two species, in particular, were abundant in certain of these habitats. Talaromyces emersonii was also widely distributed and in

natural areas showed a particular distribution in acidic soils. In the very acid pine-wood soils and the lesser acidic deciduous woodland soils it was very well represented and it may be that this species is better adapted to soils where acidic conditions predominate. It does, of course, occur in the neutral-alkaline semi-natural and industrial areas but the warm conditions may be a vital factor. Another fungus which may be restricted by pH is Chaetomium thermophile; this species was very rarely recorded from acidic soils (i.e. below 6.0) but was recorded from a number of neutral-alkaline habitats and often in substantial amounts, e.g. in the salt marsh soil it formed over 20% of the total isolations at a pH of 7.7-8.0, in a cooling tower this species also comprised over 20% of the total isolations (100% occurrence) at a pH of 7.6-7.9. The appearance of this species in composts, manures, sewage and coal spoil tips, often represented by a large number of isolates, and where the pH rarely falls below 6.5, may also be indicative of its acid sensitivity and hence its limitation to neutral-alkaline habitats.

Allescheria terrestris and Dactylomyces crustaceus were represented in a number of natural habitats, usually in small amounts, but were not recorded from manures,

composts or birds' nests where high temperatures might appear to be more conducive to their growth. Other species, viz: Absidia ramosa, Myriococcum albomyces, Sporotrichum thermophile and to some extent Talaromyces duponti, were present in a high, substantial number of all habitats but, generally, were isolated in small proportions. These species, however, had a higher occurrence in semi-natural and industrial areas relative to natural areas, i.e. they occurred in less than half the natural habitats but were present in the majority of the other habitats.

Thermoidium sulphureum showed a very limited distribution in natural areas but was present in several warm habitats. Chrysosporium sp. is another border-line species which occurred in several natural and industrial areas, and was fairly well represented when it occurred in normal soils. This species may be acid sensitive due to its strict limitation to neutral-alkaline habitats, in particular it was isolated from chalk and limestone grassland (pH 7.2-7.5) a number of times.

Group 3 species include: Absidia corymbifera, Aspergillus fumigatus (various mutants), Aspergillus nidulans, Humicola insolens, Rhizopus sp. 2 and 3 and Torula thermophila.

The mutants of A. fumigatus were present in several

normal soils and were also isolated from a number of coal spoil tips. An orange mutant occurred prolifically in a mountain heathland soil and it may be that local climatic conditions (environmental) produce and select such mutants as an evolutionary response.

Rhizopus sp. 3 was present in only one natural habitat but occurred in several of the semi-natural and industrial habitats and it may, therefore, be regarded as having a fairly limited distribution chiefly occurring in warm habitats. Rhizopus sp. 2, however, was present only in certain warm areas and, in particular, was frequently isolated from coal spoil tips where it often comprised a substantial proportion of the total isolations.

Humicola insolens was present in the majority of semi-natural areas and in a few coal spoil tips. It was particularly abundant in several of the semi-natural habitats. This species was also recorded in minute amounts from limestone grassland (pH 7.2-7.3), and it is interesting to note that Chang and Hudson (1967) reported this species as being acid sensitive due to the fact that it failed to appear on dilution plates at pH 4.5 although it was recorded by direct observation of the original material, but occurred in large numbers at pH 6.5. The

occurrence of this species in strictly neutral-alkaline habitats, as found during the present study, would tend to endorse their observations.

Torula thermophila was isolated only from mushroom compost and birds' nests but in the former habitat it was particularly prevalent, accounting for nearly 30% of the total isolations. Absidia corymbifera and Aspergillus nidulans were also restricted to a few habitats and were isolated in very small amounts.

Group 4 fungi include: Cephalosporium sp., Dactylomyces thermophilus, Humicola grisea var. thermoidea and Humicola spp. 1 and 2.

Cephalosporium sp. 1 was isolated only once from chalk grassland, Dactylomyces thermophilus was isolated from a single woodland soil and Humicola grisea var. thermoidea occurred in the non-warm area of a coal spoil tip. Humicola spp. 1 and 2 were isolated several times from a single manure heap which possessed high internal temperatures. All these species were isolated in minute amounts and would, therefore, appear to have a limited distribution and occurrence.

The remaining species of this group are represented by those fungi unique to the coal spoil tips studied.

These species can be subdivided into two groups, viz:

a. those species which occurred on several coal spoil tips, and which usually represented a substantial proportion of the total isolations, e.g. Mortierella sp., Rhizopus sp. 1, Scolecobasidium sp. and Trichophaea sp.

b. those species which were unique to one particular coal spoil tip and occurred in minute amounts, e.g. several Aspergillus species, Penicillium spp. 1 and 2, Scopulariopsis sp., Tritirachium sp. and an unidentified Hyphomycete.

These two sections can be further subdivided into those species which were isolated exclusively from the warm areas of coal spoil tips and those species which were generally distributed. However, these particular groupings have been elucidated previously in the general discussion of coal spoil tip results.

In concluding this discussion, it must be pointed out that the isolation method employed does not give a true picture of the soil microflora as it fails to distinguish between the active fungus mycelium and dormant spores of the fungus. However, during the sampling of normal soils etc., it was expected that very few of the thermophilous

fungi would be active, due to the low soil temperatures at the time of sampling. This consideration would apply to true thermophiles which probably have ephemeral life cycles. Johnson (1957) reviews the work of several authors concerning the distribution of thermophilic bacteria in the Arctic, in deep ocean-bottom cores, as well as their widespread distribution in soils where the temperature is generally below the minimum for their cultivation in the laboratory. Temperature relationships of growth, however, have been shown to be modified by nutritional factors and the author states that the growth of thermophiles may occur very slowly at temperatures lower than in artificial media. A fungus may, therefore, grow at low temperatures under certain conditions in nature, such as pressure of competition, or presence or absence of certain chemicals, but may only grow at higher temperatures with the usual methods of laboratory cultivation. Furthermore, he quotes examples of the adaptability of micro-organisms to changing environmental conditions over a short period of time. Sporovibrio desulphuricans has been shown to posses halophytic characteristics at ordinary temperatures but on normal media grows as a thermophile, these two properties are reversible. Therefore, organisms isolated

by selective factors, as in the present study, may be unable to grow in nature with same characterisitics. Certain flagellates have been shown to acclimatise, over a period of seven years, to temperatures of 70°C instead of their usual optimum temperatures of about 15°C, and were unable to grow when transferred to these low temperatures. But this is a relatively long period of adaptation compared with some bacteria, and perhaps fungi. The presence of thermophiles in soils and other unusual habitats can, therefore, partly be explained and it is possible that adaptation by fungi to the warm conditions which exist in certain industrial habitats, (e.g. coal spoil tips), may occur, given sufficient time.

As previously discussed, the thermophilous fungi which occur in normal soils may be adapted to short periods of high temperature during the summer months, when rapid growth and sporulation may occur. The fast growth rate of some of these species at higher temperatures may be an added survival value in the colonisation of new micro-habitats. Apinis (1960) located clusters of mycelia, gemmae and perithecial initials of thermophilous fungi in the plant debris of coastal grasslands. These structures may secure the survival of these fungi during unfavourable

conditions. The high proportion of Ascomycetes and other fungi capable of producing resistant overwintering structures (e.g. Phycomycetes) which was found to constitute the thermophilous fungal populations of normal soils, and to some extent warm areas, during the present study, may be due to their ability to survive the long periods when conditions are unfavourable for their growth and then to grow rapidly during more optimum conditions. Previous reports of the mesophilic populations of similar habitats have recorded a dominance of Fungi Imperfecti and relatively few Ascomycetes (Warcup 1951).

The use of selective media and different isolation methods has been shown by Chesters and Thornton (1956) to have a profound effect on the variety of fungal species isolated from a single soil type. The fact that during the present study only one method of isolation was used and only one isolation medium in general was employed, may have biased the results to some extent. Although other media such as Czapek-Dox, corn meal and yeast-starch agars were used in the isolations from certain coal spoil tips, no significant quantitative or qualitative differences were found. The selective effect of temperature may, to some extent, cancel out the other errors introduced during isolation.

CHAPTER III

SEASONAL DISTRIBUTION

AND

PROFILE STUDIES

The seasonal distribution of thermophilic and thermotolerant fungi was studied from two different aspects. Firstly, the presence of these fungi was investigated in a selected habitat, in this case a coal spoil tip, and secondly an investigation of the thermophilic fungal air spora was undertaken. Both these projects were carried out over a twelve monthly period together with measurements of the environmental conditions.

i. Seasonal distribution in a coal spoil tip profile

Due to the fact that certain areas of Leycett coal spoil tip have been undisturbed for a number of years and, therefore, have been open to colonisation by fungi and higher plants, a stable habitat in close proximity to the University was immediately available. This close proximity was considered to be especially important in view of the frequent air spora measurements to be taken. The sampling area selected is situated on one of the subsidiary tips which are colonised by Betula verrucosa and a number of wasteland weeds (see Plate 7). The absence of unusually high 'soil' temperatures (i.e. "warm areas") was a specific requirement as these, and not the

a. General view of sampling area.

Plate 7 Leycett spoil tip: area of profile study

b. Close up to show coarse, grey shale and thin topsoil, Cladonia sp. in foreground.

seasonal factors, may influence the distribution of thermophilic fungi. Samples were taken twice a month for a full year - March 1968 until February 1969.

Sampling procedure

A pit was dug approximately one foot square and about one foot in depth. Below this level coal and shale was present together with areas of yellow sulphur deposits, making it an unsuitable habitat for fungal growth. In fact, samples initially analysed from below 25 cms. (approximately 10 ins.) showed no thermophilic fungal growth. In view of this preliminary investigation samples were taken from the top-soil, which included substantial amounts of humus, and from varying depths below this layer, viz: 2.5-5 cms., 10-15 cms., 20-25 cms. Before sampling, the walls of the pit were scraped with a sterilised spatula (flamed in 95% ethanol) to prevent contaminating material from other layers being collected; this contamination would occur during the initial digging of the pit. At each designated sampling level a composite sample was taken from each wall of the pit in a sterile polypropylene collecting bottle. At each sampling date two profiles were investigated and air

temperature and soil temperature were correspondingly recorded.

The pH was measured in the laboratory using the glass electrode meter method. The percentage water content of each sample was also measured by weighing the fresh sample which was then oven dried at 120°C to constant weight. The isolation methods were those previously described (see Chapter I) but a slight variation was introduced concerning incubation temperature. Half the plates were incubated at 45°C and the remainder at 50°C, in order to determine whether or not the slight incubation temperature difference would affect the number and variety of species recorded.

Results and Discussion

These are presented as a monthly figure for each sample level (horizon) and are composed of the results of two separate sampling dates and two separate profiles. This gives a total of sixteen plates per month for each horizon studied. For the complete results see Tables 21 to 26.

Several interesting species were recorded during this profile study which have not previously been isolated, viz: *Cephalosporium* sp. 2, *Chaetomium* spp. 1 and 2, *Geotrichum* sp. and *Talaromyces* sp. The taxonomy

Table 21

Leycett Coal Spoil Tip - Monthly Assay for March and April

Species	March								April							
	topsoil		2.5 - 5 cms.		10 - 15 cms.		20 - 25 cms.		topsoil		2.5 - 5 cms.		10 - 15 cms.		20 - 25 cms.	
	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.
Mucor pusillus	9	18.7	2	12.5	5	18.7	2	12.5	36	50.0	-	-	-	-	-	-
Mucor sp.	2	6.2	-	-	-	-	-	-	4	12.5	1	6.2	2	12.5	-	-
Rhizopus sp. 2	2	6.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allescheria terrestris	7	25.0	-	-	3	12.5	-	-	-	-	-	-	-	-	-	-
Chaetomium thermophile var. dissitum	2	12.5	1	6.2	-	-	-	-	8	25.0	1	6.2	-	-	-	-
Myriococcum albomyces	1	6.2	-	-	-	-	-	-	-	-	5	25.0	-	-	-	-
Talaromyces sp.	2	12.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thermoascus aurantiacus	10	25.0	6	18.7	-	-	1	6.2	10	18.7	-	-	-	-	-	-
Aspergillus fumigatus	81	87.5	23	68.7	51	87.5	17	43.7	18	25.0	4	12.5	-	-	-	-
Geotrichum sp.	2	6.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sporotrichum thermophile	4	18.7	4	25.0	-	-	-	-	-	-	-	-	-	-	-	-
Thermomyces lanuginosus	26	75.0	5	18.7	11	50.0	12	31.2	42	62.5	-	-	3	12.5	-	-
Total isolations	148		41		70		32		118		11		5		0	
Number of species	12		6		4		4		6		4		2		0	

Table 22

Leycett Coal Spoil Tip - Monthly Assay for May and June

Species	May								June							
	topsoil		2.5 - 5 cms.		10 - 15 cms.		20 - 25 cms.		topsoil		2.5 - 5 cms.		10 - 15 cms.		20 - 25 cms.	
	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.
Absidia ramosa	9	25.0	-	-	1	6.2	-	-	-	-	-	-	-	-	-	-
Mucor puillus	19	31.2	11	18.7	9	25.0	-	-	36	56.2	5	18.7	-	-	-	-
Rhizopus sp. 1	6	12.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allescheria terrestris	2	6.2	13	25.0	8	25.0	-	-	3	12.5	-	-	5	18.7	-	-
Chaetomium thermophile var. dissitum	-	-	-	-	5	12.5	2	6.2	5	25.0	-	-	3	12.5	1	6.2
Myriococcum albomyces	-	-	5	12.5	2	6.2	-	-	3	6.2	1	6.2	-	-	-	-
Talaromyces duponti	-	-	3	12.5	1	6.2	-	-	-	-	-	-	-	-	-	-
Talaromyces sp.	-	-	-	-	19	18.7	-	-	-	-	-	-	5	12.5	-	-
Talaromyces emersonii	5	12.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thermoascus aurantiacus	23	37.5	14	25.0	8	12.5	2	6.2	18	31.2	33	50.0	5	18.7	2	6.2
Aspergillus fumigatus	27	25.0	16	56.2	21	43.7	2	6.2	45	68.7	16	25.0	6	18.7	-	-
Sporotrichum thermophile	-	-	-	-	-	-	4	12.5	4	18.7	-	-	-	-	-	-
Thermoidium sulphureum	-	-	3	12.5	-	-	-	-	-	-	-	-	-	-	-	-
Thermomyces lanuginosus	15	37.5	-	-	14	37.5	-	-	9	12.5	11	25.0	-	-	-	-
Total isolations	106		65		88		10		123		66		24		3	
Number of species	8		7		10		4		8		5		5		2	

Table 23

Leycett coal spoil tip - monthly assay for July and August

Species	July								August							
	topsoil		2.5 - 5 cms.		10 - 15 cms.		20 - 25 cms.		topsoil		2.5 - 5 cms.		10 - 15 cms.		20 - 25 cms.	
	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.
Absidia corymbifera	1	6.2	-	-	-	-	-	-	-	-	-	-	2	6.2	-	-
Absidia ramosa	13	31.2	2	6.2	-	-	1	6.2	-	-	-	-	-	-	-	-
Mucor pusillus	43	62.5	-	-	3	6.2	-	-	10	25.0	11	25.0	9	18.7	-	-
Mucor sp.	1	6.2	-	-	-	-	-	-	5	12.5	-	-	-	-	-	-
Rhizopus sp. 1	9	12.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allescheria terrestris	-	-	8	25.0	6	12.5	-	-	-	-	-	-	-	-	-	-
Chaetomium thermophile var. dissitum	4	12.5	13	25.0	-	-	-	-	8	25.0	-	-	2	6.2	1	6.2
Myriococcum albomyces	-	-	-	-	1	6.2	-	-	-	-	3	12.5	-	-	-	-
Thermoascus aurantiacus	-	-	5	12.5	3	6.2	-	-	6	18.7	2	12.5	-	-	-	-
Trichophaea sp.	-	-	-	-	2	12.5	-	-	-	-	-	-	-	-	-	-
Aspergillus fumigatus	96	81½2	82	62.5	3	12.5	7	18.7	123	100	68	75.0	63	68.7	13	25.0
Chrysosporium sp.	1	6.2	-	-	-	-	-	-	-	-	8	12.5	-	-	-	-
Geotrichum sp.	-	-	2	12.5	1	6.2	-	-	-	-	-	-	-	-	-	-
Humicola insolens	1	6.2	1	6.2	-	-	-	-	-	-	1	6.2	-	-	-	-
Sporotrichum thermophile	38	50.0	29	37.5	-	-	2	6.2	-	-	4	18.7	9	25.0	-	-
Thermoidium sulphureum	2	6.2	5	25.0	-	-	-	-	-	-	-	-	-	-	-	-
Thermomyces lanuginosus	61	87.5	20	37.5	2	6.2	-	-	104	93.7	31	50.0	-	-	-	-
Total isolations	270		167		21		10		256		128		85		14	
Number of species	12		10		8		3		6		8		5		2	

Table 24

Leycett Coal Spoil Tip - Monthly Assay for September and October

Species	September								October							
	topsoil		2.5 - 5 cms.		10 - 15 cms.		20 - 25 cms.		topsoil		2.5 - 5 cms.		10 - 15 cms.		20 - 25 cms.	
	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.
Mucor pusillus	6	12.5	-	-	-	-	-	-	24	56.2	2	12.5	-	-	-	-
Mucor sp.	-	-	-	-	-	-	-	-	2	6.2	-	-	-	-	-	-
Allescheria terrestris	-	-	-	-	-	-	-	-	6	25.0	1	6.2	-	-	-	-
Chaetomium thermophile var. coprophile	-	-	3	6.2	-	-	-	-	4	12.5	-	-	-	-	-	-
Chaetomium thermophile var. dissitum	-	-	4	12.5	-	-	-	-	12	25.0	-	-	-	-	-	-
Dactylomyces crustaceus	3	12.5	2	12.5	-	-	-	-	-	-	-	-	-	-	-	-
Talaromyces duponti	1	6.2	2	6.2	3	12.5	-	-	2	6.2	-	-	-	-	-	-
Talaromyces sp.	8	37.5	3	6.2	-	-	-	-	-	-	-	-	-	-	-	-
Talaromyces emersonii	1	6.2	6	12.5	-	-	-	-	2	6.2	1	6.2	-	-	-	-
Thermoascus aurantiacus	10	25.0	3	6.2	-	-	2	6.2	4	12.5	6	12.5	-	-	-	-
Aspergillus fischeri	1	6.2	1	6.2	-	-	-	-	-	-	-	-	-	-	-	-
Aspergillus fumigatus	7	25.0	3	12.5	3	18.7	2	6.2	31	31.2	16	25.0	9	18.7	-	-
A. fumigatus: orange var	2	6.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. nidulans	-	-	2	12.5	-	-	-	-	-	-	-	-	-	-	-	-
Humicola grisea var. thermoidea	-	-	2	6.2	-	-	-	-	-	-	-	-	-	-	-	-
Sporotrichum thermophile	-	-	-	-	-	-	-	-	4	18.7	2	6.2	4	12.5	-	-
Thermoidium sulphureum	-	-	-	-	-	-	-	-	-	-	2	6.2	-	-	-	-
Thermomyces lanuginosus	9	25.0	-	-	-	-	-	-	47	50.0	9	25.0	2	12.5	2	6.2
Total isolations	48		31		6		4		138		39		15		2	
Number of species	10		11		2		2		11		8		3		1	

Table 25

Leycett Coal Spoil Tip - Monthly Assay for November and December

Species	November								December							
	topsoil		2.5 - 5 cms.		10 - 15 cms.		20 - 25 cms.		topsoil		2.5 - 5 cms.		10 - 15 cms.		20 - 25 cms.	
	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.
Absidia ramosa	1	6.2	-	-	-	-	1	6.2	14	43.7	2	6.2	-	-	-	-
Mucor pusillus	32	81.2	8	25.0	1	6.2	-	-	47	93.7	2	12.5	-	-	-	-
Rhizopus sp. 1	3	6.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhizopus sp. 3	2	6.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allescheria terrestris	6	25.0	4	18.7	-	-	-	-	3	18.7	18	50.0	24	37.5	2	12.5
Chaetomium thermophile var. coprophile	4	18.7	-	-	-	-	-	-	2	6.2	-	-	-	-	-	-
var. dissitum	2	12.5	5	12.5	6	18.7	-	-	1	6.2	2	12.5	3	12.5	-	-
Chaetomium sp. 1	1	6.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chaetomium sp. 2	-	-	-	-	-	-	-	-	1	6.2	-	-	-	-	-	-
Dactylomyces crustaceus	-	-	1	6.2	-	-	-	-	-	-	-	-	1	6.2	-	-
Myriococcum albomyces	-	-	5	25.0	2	12.5	-	-	-	-	8	37.5	6	25.0	1	6.2
Talaromyces duponti	-	-	-	-	1	6.2	-	-	-	-	-	-	1	6.2	-	-
Talaromyces sp.	-	-	-	-	-	-	-	-	-	-	5	12.5	-	-	-	-
Thermoascus aurantiacus	3	12.5	2	6.2	-	-	-	-	1	6.2	4	18.7	-	-	-	-
Aspergillus fumigatus	96	100	52	81.2	27	62.5	3	12.5	92	87.5	43	75.0	24	37.5	3	18.7
Chrysosporium sp.	3	12.5	2	6.2	-	-	-	-	-	-	1	6.2	4	12.5	-	-
Geotrichum sp.	-	-	-	-	-	-	-	-	-	-	1	6.2	-	-	1	6.2
Humicola grisea var. thermoidea	1	6.2	-	-	1	6.2	-	-	-	-	-	-	4	12.5	-	-
Humicola insolens	2	6.2	1	6.2	2	6.2	1	6.2	-	-	8	12.5	-	-	-	-
Sporotrichum thermophile	2	12.5	7	25.0	5	31.2	1	6.2	5	18.7	6	25.0	1	6.2	-	-
Thermoidium sulphureum	-	-	2	6.2	-	-	-	-	-	-	1	6.2	-	-	-	-
Thermomyces lanuginosus	68	93.7	27	62.5	1	6.2	-	-	69	93.7	8	31.2	-	-	-	-
Total isolations	226		116		46		6		235		109		68		7	
Number of species	15		12		9		4		10		14		9		4	

Table 26

Leycett Coal Spoil Tip - Monthly Assay for January and February

Species	January								February							
	topsoil		2.5 - 5 cms.		10 - 15 cms.		20 - 25 cms.		topsoil		2.5 - 5 cms.		10 - 15 cms.		20 - 25 cms.	
	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.	no.	% occ.
Absidia ramosa	23	50.4	4	25.0	-	-	-	-	2	12.5	4	25.0	-	-	-	-
Mucor pusillus	71	93.7	6	25.0	1	6.2	-	-	51	100	11	37.5	-	-	-	-
Rhizopus sp. 1	3	6.2	-	-	-	-	-	-	8	18.7	-	-	-	-	-	-
Allescheria terrestris	-	-	7	31.2	-	-	-	-	2	6.2	31	50.0	8	25.0	-	-
Chaetomium thermophile var. coprophile	1	6.2	4	18.7	-	-	-	-	1	6.2	-	-	-	-	-	-
var. dissitum	-	-	6	18.7	3	6.2	-	-	5	18.7	9	18.7	2	6.2	-	-
var. thermophile	2	6.2	3	6.2	-	-	-	-	-	-	1	6.2	2	6.2	-	-
Dactylomyces crustaceus	-	-	-	-	-	-	-	-	2	6.2	1	6.2	-	-	-	-
Myriococcum albomyces	-	-	6	25.0	2	12.5	8	37.5	-	-	2	12.5	9	37.5	1	6.2
Talaromyces emersonii	2	6.2	-	-	-	-	-	-	-	-	2	12.5	-	-	-	-
Talaromyces sp.	-	-	-	-	-	-	-	-	-	-	17	25.0	1	6.2	-	-
Thermoascus aurantiacus	-	-	-	-	1	6.2	-	-	1	6.2	3	12.5	1	6.2	-	-
Aspergillus fumigatus	95	93.7	98	100	27	87.5	20	43.7	65	68.7	53	81.2	24	68.7	1	6.2
A. fumigatus: brown var.	2	6.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cephalosporium sp. 2	-	-	-	-	-	-	-	-	-	-	2	6.2	-	-	-	-
Chrysosporium sp. 1	3	12.5	6	25.0	-	-	-	-	2	6.2	2	6.2	-	-	-	-
Geotrichum sp.	-	-	2	6.2	-	-	-	-	-	-	-	-	2	12.5	-	-
Humicola grisea var. thermoidea	-	-	-	-	2	6.2	-	-	-	-	-	-	-	-	-	-
Sporotrichum thermophile	-	-	12	37.5	2	12.5	1	6.2	-	-	6	18.7	1	6.2	-	-
Thermomyces lanuginosus	75	100	13	50.0	-	-	-	-	81	100	19	43.7	-	-	-	-
Total isolations	277		167		38		29		220		163		50		2	
Number of isolations	10		12		7		3		11		15		9		2	

of these species is discussed in Chapter V. Two of the species, Geotrichum sp. and Talaromyces sp., were consistently isolated from the various layers of the coal spoil tip profile and would appear to be a stable part of the thermophilous fungal population of this spoil tip. Other species previously isolated exclusively from coal spoil tips, and in particular from the warm areas, were sporadically isolated, viz: Rhizopus sp. 1 and Trichophaea sp. When slightly lower incubation temperatures were employed, significant differences were not apparent, although Aspergillus fischeri was recorded for the first time only on plates incubated at 45°C.

Graphical analyses of the effect of season and increasing depth on the thermophilous fungal population are presented in Figures 6 and 7. From Figure 6 it can be seen that the number of species recorded generally decreases with depth, although frequently in the horizon just below the topsoil a greater number of species occurred than was present in the topsoil. There would appear, however, to be a significant decrease in the number of species isolated at the lower layers, in particular at the deepest horizon (20-25 cms.) where few species were recorded, never more than four species being present.

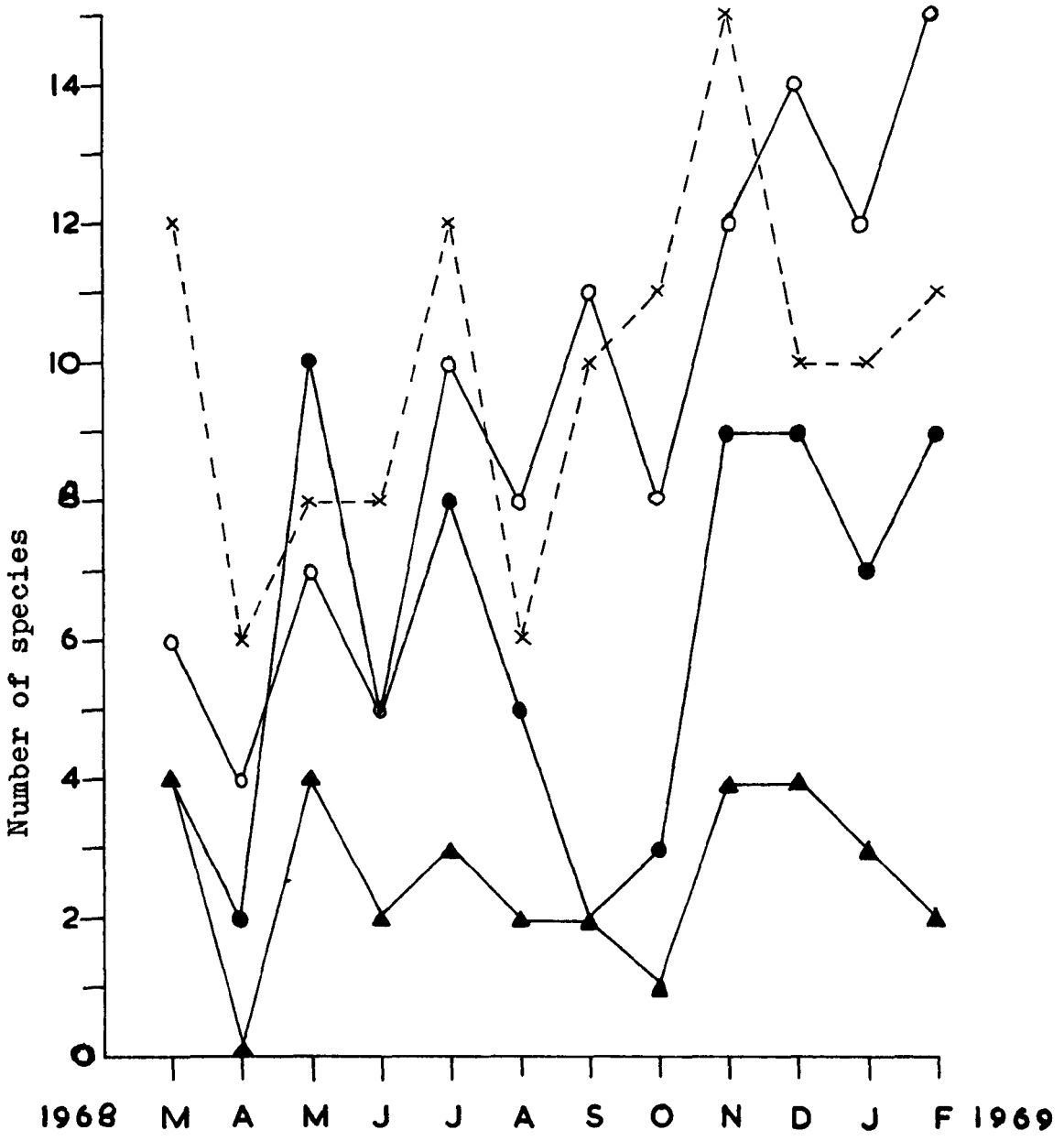


Figure 6

Number of species present in the monthly assay of each coal spoil tip horizon viz: topsoil (x---x), 2.5-5cm.(o—o), 10-15cm.(●—●), and 20-25cm.(▲—▲).

Regarding the seasonal variation in species numbers, there would appear to be consistent peaks during July and again from November to December, and high numbers were also recorded during February. This is particularly clear from the results of the upper three horizons which show comparable increases during these periods. In general, the numbers of species isolated from the lowest layer do not reflect the variation present in the other layers and seasonal factors would not appear to have a significant effect.

The number of isolates recorded also fell markedly with increase in depth (see Figure 7). The numbers isolated from the topsoil were consistently in excess of those recorded from any of the other horizons, below this layer there was a progressive decrease in isolate numbers. Very few isolates were obtained from the lower two layers, and in particular from the deepest horizon, where numbers were almost negligible. Seasonal variations in the number of isolates were most marked in the upper layers of the profile and the lower two horizons were less obviously affected. The isolate numbers recorded rose during the early summer and peaks were reached during July and August, when soil temperatures (solar insolation) are high. During the autumn months

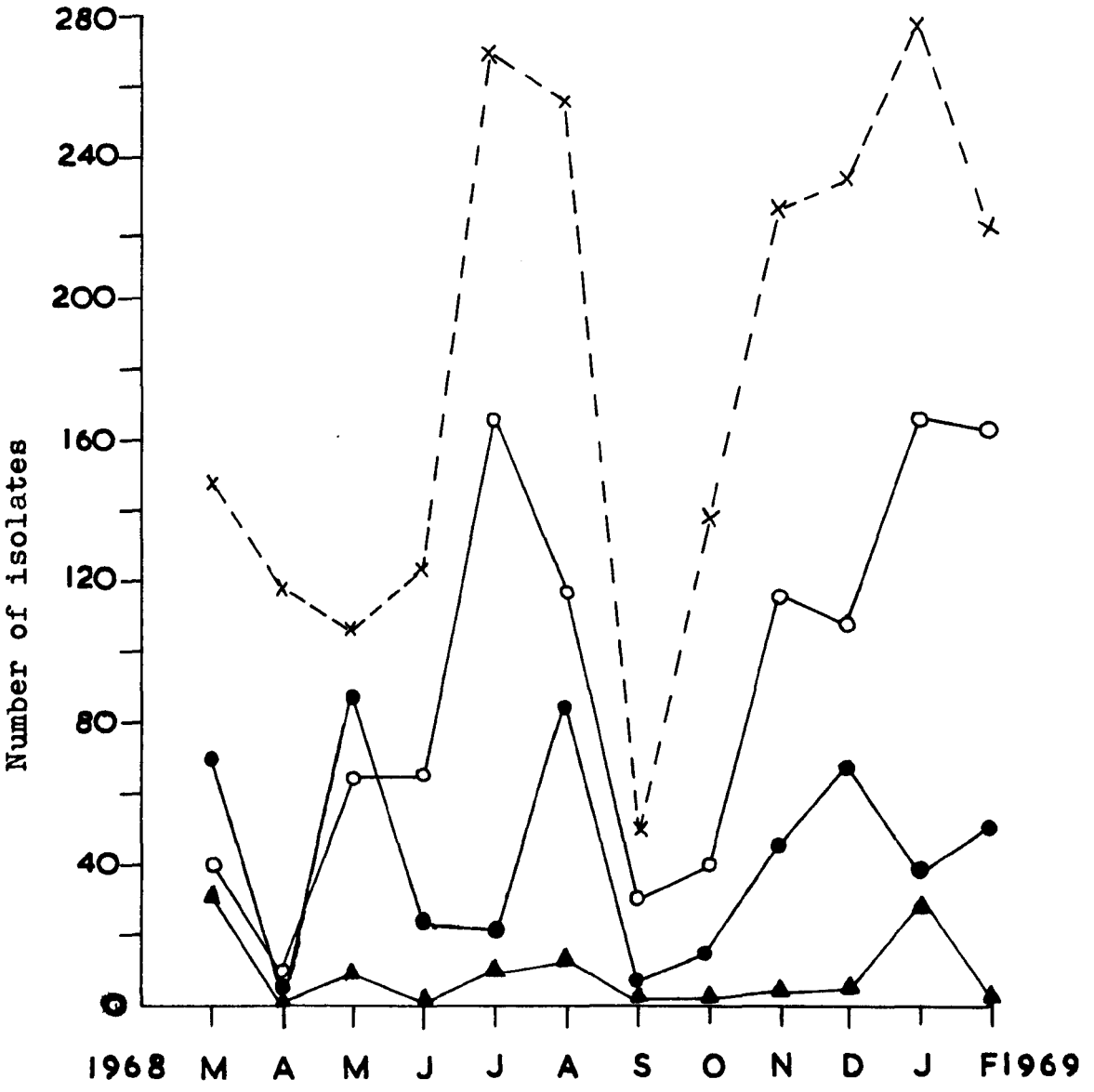


Figure 7

Number of isolates recorded from the monthly assay of each coal spoil tip horizon viz: topsoil (x---x), 2.5-5cm.(o—o), 10-15cm.(●—●) and 20-25cm.(▲—▲).

numbers fell dramatically and this was apparent at all levels of the profile. Suprisingly an increase in isolate numbers occurred during November and up to February, reaching a peak in January, i.e. a period when soil temperatures are at a minimum.

The environmental factors most likely to affect the thermophilous fungal populations (and fungi in general) are presented in Table 27. From this table it can be seen that the pH increases with depth and at the lower layers conditions may be strongly alkaline. The vegetation present in the topsoil will tend to impart an acidic nature to this layer, however, the almost complete absence of organic matter at the lower levels results in very alkaline conditions reflecting the general basic properties of coal spoil material. From October to February the topsoil showed a progressive increase in acidity and this may be a consequence of leaf-fall which would greatly increase the organic matter content of the upper layers of the profile. This may also explain the marked increase in the numbers of fungi recorded during these months. The soil moisture content decreases rapidly with increasing depth and at the lower levels the water content recorded during June until September

Table 27

Monthly pH and soil moisture measurements with soil and air temperature recordings

Month	Temp. °C.		topsoil		2.5-5cms.		10-15cms.		20-25cms.	
	Soil*	air	pH	% H_2O	pH	% H_2O	pH	% H_2O	pH	% H_2O
Spring										
March	8	7.5	6.4	42.6	7.0	22.3	7.4	16.9	7.6	15.2
April	14	15	6.4	50.8	6.8	28.6	7.1	17.3	7.5	15.8
May	16.5	16	6.6	34.3	7.1	24.5	7.4	15.1	7.6	12.4
Summer										
June	21	22	5.9	11.2	6.2	11.8	6.7	9.4	7.5	8.9
July	20.5	21.5	6.6	12.1	7.0	11.0	7.0	8.5	7.6	6.7
August	17.5	18	6.3	20.6	7.3	17.8	7.6	12.1	7.9	11.4
Autumn										
September	17	17.5	6.2	22.3	6.6	21.5	7.3	14.0	7.7	13.1
October	15	16	5.6	26.8	6.5	16.4	7.1	14.9	7.2	13.7
Winter										
November	8	9.5	5.7	41.5	6.6	20.5	7.0	16.1	7.4	16.4
December	4	5	5.6	45.1	6.2	27.2	6.5	18.3	6.5	17.0
January	4.5	6	5.5	50.2	6.3	17.4	6.8	12.3	6.9	11.5
February	2	2	5.3	45.3	5.8	27.7	6.5	16.1	6.6	12.0

* at a depth of approximately 5 cms.

may be a significant factor in determining the size of the thermophilous fungal population, although the subsequently high surface temperatures may be thought to produce a marked increase in the numbers of thermophilous fungi present. There may be, therefore, a delicate balance between the amount of soil moisture present and the occurrence of sufficiently high soil temperatures to encourage the thermophilous growth habit. As previously noted, variations in the numbers of thermophilous fungal species and isolates cannot be satisfactorily correlated with seasonal factors affecting soil temperature. It would appear that the thermophilous fungal population is affected more by organic matter content or soil moisture content than by soil temperature. This would appear to be anomalous on account of the strict dependence of thermophilous fungi on the presence of elevated temperatures in the environment, in order to encourage mycelial growth. The consistent increase in the numbers of thermophilous fungal species and isolates during the winter months may be due to the occurrence of leaf-fall during the previous autumn. This significant increase in the organic matter content, especially in the upper surface layers, may lead to increased microbial

activity and the possible creation of local pockets of increased temperature sufficient for the growth of the thermophilous fungal population. The relatively low numbers of thermophilous fungi recorded during certain warmer months of the year, in particular during September, may be due to low soil moisture and organic matter content. Tresner et al. (1954) found that in a forest soil, temperature played only a minor role in determining the size of the fungal populations, since the winter populations were always greater than those in summer.

The high pH present in the lower layers of the coal spoil tip profile may be important in eliminating certain species, and this has been shown by Parkinson and Balasooriya (1967) in an investigation of a pine-wood soil profile. Fungal species were found to favour acidic conditions and to be markedly affected by a high pH. As discussed earlier (see Chapter II) the amount of available nitrogen is usually restricted in alkaline spoil material and this condition together with the low soil moisture and organic matter content which exist in the lower horizons, plus the reduced oxygen levels and increased carbon dioxide concentrations, may explain the very small numbers of species and isolates recorded from

these layers (i.e. 10-25 cms.). The possible increase in sulphur concentrations at the lower levels may also affect these numbers. It would appear that this marked effect of increasing depth on the thermophilous fungal population begins to operate after a relatively short distance.

Apinis (1960) reported that the numbers of thermophilous micro-organisms in the soil show a distinct seasonal variation. In particular, thermophilous fungi were found to occur in the soil at higher frequencies in autumn and winter, but were at their lowest in the spring. As can be seen from Tables 21 and 22 (see also Figure 7) the numbers of thermophilous fungal isolates recorded during March, April and May were particularly low, but showed a significant rise during July and August (i.e. late summer). In the autumn months, however, these numbers fell sharply, and then escalated rapidly during the winter months. In conclusion, it can be said that seasonal variation did occur and that high numbers of thermophilous isolates (and species) were obtained during the late summer and winter months; distinct minima occurred during some of the autumn and spring months. This rise in numbers during the winter

months is difficult to correlate with the low air and soil temperatures recorded over this period (see Table 27).

From a qualitative aspect there would appear to be seasonal and depth variation. Absidia ramosa and Mucor pusillus were isolated more frequently during the winter months than during the summer and autumn months. Apinis (1963b) in a study of coastal grasslands reported a similar phenomenon, namely that Absidia ramosa, Mucor pusillus and Sporotrichum thermophile were more common in samples collected during winter than in those at other seasons of the year. In the present study, however, Sporotrichum thermophile did not show this distribution and in fact was generally more prevalent during the summer months. Aspergillus fumigatus was generally consistently isolated during the twelve month period, usually in large numbers, and it was little affected by seasonal change, although relatively low levels were recorded during September and October. Thermomyces lanuginosus was also infrequently isolated in autumn, as well as during the spring and early summer months. This species was, however, isolated frequently during July and August and again during the winter months. Few of

the other species can be said to show a definite seasonal variation as they generally occurred sporadically throughout the sampling period, and Allescheria terrestris, Thermoascus aurantiacus and to a certain extent Chaetomium thermophile were isolated consistently. Other species were isolated only during the winter months and were represented by a few isolates, e.g. Cephalosporium sp. 2, Chaetomium spp. 1 and 2.

Aspergillus fumigatus was the only member of the common species group to occur regularly in the lower layers of the soil profile. The other members of this group, Mucor pusillus, and Thermomyces lanuginosus, were generally present only in the upper layers and this may reflect their intolerance of the environmental conditions existing in the lower horizons. Conversely, certain species such as Chaetomium thermophile and Myriococcum albomyces were usually well represented in the lower layers of the profile and were often the dominant species. These species may be better suited to the conditions present in the lower horizons, especially to the high pH (and this was initially discussed in Chapter II), and their relative frequency may be an indication of the reduced competition. Myriococcum albomyces was also of

relatively common occurrence in sand-dune systems and it may be that this species is able to tolerate conditions of high pH and low soil moisture content. This may also apply to Aspergillus fumigatus and to a certain extent Allescheria terrestris and Thermoascus aurantiacus, which regularly occurred in samples taken from the lower horizons. The Phycomycetes were poorly represented in the lower layers and occurred chiefly in the topsoil.

The high proportion of Ascomycetes isolated during this profile study, especially at the lower levels, may reflect the ability of these species to survive the unfavourable conditions by the production of resistant sexual structures. This may, of course, account for their occurrence during the winter months, the colonies on the soil plates resulting from the germination of ascospores. This is, as mentioned earlier, one of the faults of the soil plate method as it fails to give a true picture of the soil fungal population, i.e. there is no differentation between actively growing mycelium and spore stages.

ii. Air Spora

A simple spore trap was designed (see Diagram 1a) for use at appropriate sample areas. The apparatus consisted of an adjustable platform containing apertures for two petri plates. A cover was provided and the platform could be adjusted in height and elevation to face directional winds or avoid direct flooding by rain etc. Two models were constructed, one designed to operate at ground level and the other designed to sample the air spora three to four feet above the ground, and for this purpose was provided with extendable legs which were secured into the substratum by means of long steel tips.

The original intention was to sample the air spora of the area in which 'soil' profile studies were being undertaken, i.e. Leycett coal spoil tip, and thus compare the seasonal distribution of thermophilous fungi in the 'soil' and in the air of one area. However, the spore trap was tampered with and damaged by children during preliminary measurements and the idea had to be abandoned. It was finally decided to fix one of the models on the roof of the Keele Biology Department, (approximately three miles from Leycett), approximately

a) Stationary

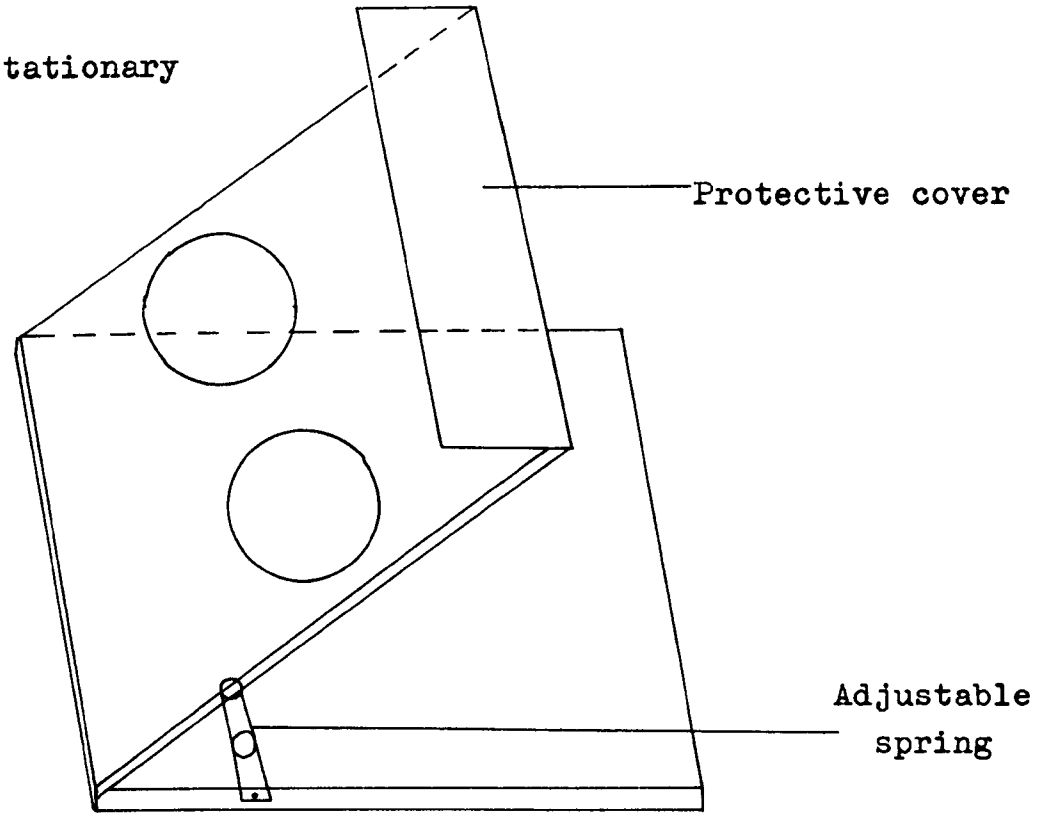
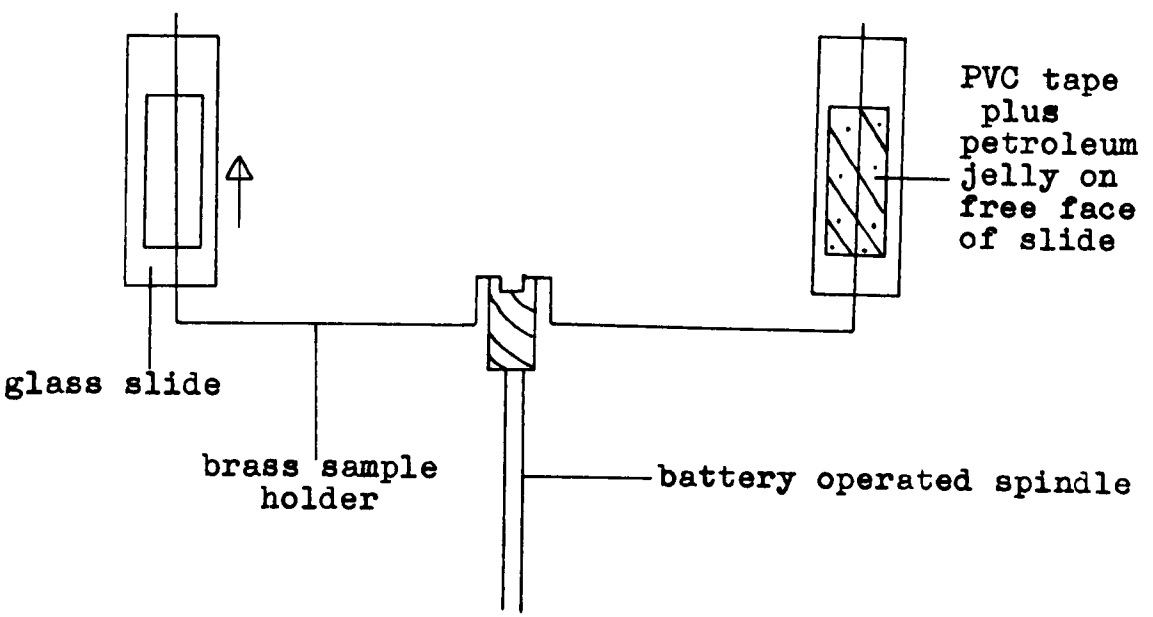


Diagram 1 Spore Traps

b) Mechanical



sixty feet above the ground. Trial petri plate exposures were conducted and the optimum time of exposure, in order to obtain good colony counts, was determined. Each plate was exposed for eight hours, from approximately 10 a.m. until 6 p.m. for five days per week, and only in particularly bad weather conditions were measurements curtailed. An average of thirty-six to forty plates per month were exposed, from March 1968 until February 1969. In order to prevent excessive desication of the agar, very deep agar plates were poured and only on the hottest of days was appreciable drying-out encountered. In this situation the plates were replaced after four hours and the two results were equivalent to that of an eight hour sample. Potato dextrose agar was used as the medium for all sample plates, which were incubated at 45°C.

A mechanical spore trap was also tested for use at sampling areas (see Diagram 1b). A battery operated spindle was used to drive a brass sample holder. The latter consisted of a length of brass wire attached to the spindle with a sampling arm at either end. To each sampling arm a glass slide was affixed by means of cellotape with the free face exposed to the direction of movement of the arm. To this free face a small strip of

white PVC tape was attached, the outer surface of which was coated with a layer of petroleum jelly. The brass holder, glass slide, petroleum jelly and tape were previously autoclaved at 10lb/in^2 for ten minutes and the apparatus set up using sterile forceps and scissors in a sterile incubating room. A number of sample holders were prepared before each sampling day and stored in sterile polypropylene field boxes. At an appropriate area a sample holder was attached to the spindle which was then operated for five minutes at about three to four feet above the ground. The sample holder was then stored and later analysed in the laboratory. The strip of cellotape plus petroleum jelly was removed under aseptic conditions and transferred to a plate of potato dextrose agar and incubated at 45°C.

Unfortunately few fungal species were isolated by this method, viz: Aspergillus fumigatus, Mucor pusillus, and Thermomyces lanuginosus, and these only in very small numbers. The time of exposure and running of the sample holder was considered to be critical but it was not practically possible to extend the time by any appreciable extent. Furthermore, exposure of petri plates for ten to fifteen minutes during these sampling periods

failed to record any thermophilous fungal species. It can be assumed, therefore, that the thermophilic fungal air spora is not well represented at any sampling area and can only be demonstrated consistently by long exposure time (i.e. eight hours). Daily air spora measurement was therefore considered to be of practicable feasibility only in the immediate vicinity of the laboratory. Hence, the thermophilic air spora samplings are presented from the results recorded from the spore trap situated on the Keele Biology Department roof.

Results and Discussion

The average number of colonies recorded per day and the total number of species isolated per month are presented graphically in Figure 8.

The number of colonies recorded was initially low during the early spring but rose gradually during the summer months and reached a peak in July and August; this was followed by a sharp decline and low numbers of thermophilous fungal isolates were recorded during the autumn. There was, however, a marked rise during the winter months and a peak was reached in January, tailing off somewhat during February. Correspondingly, the

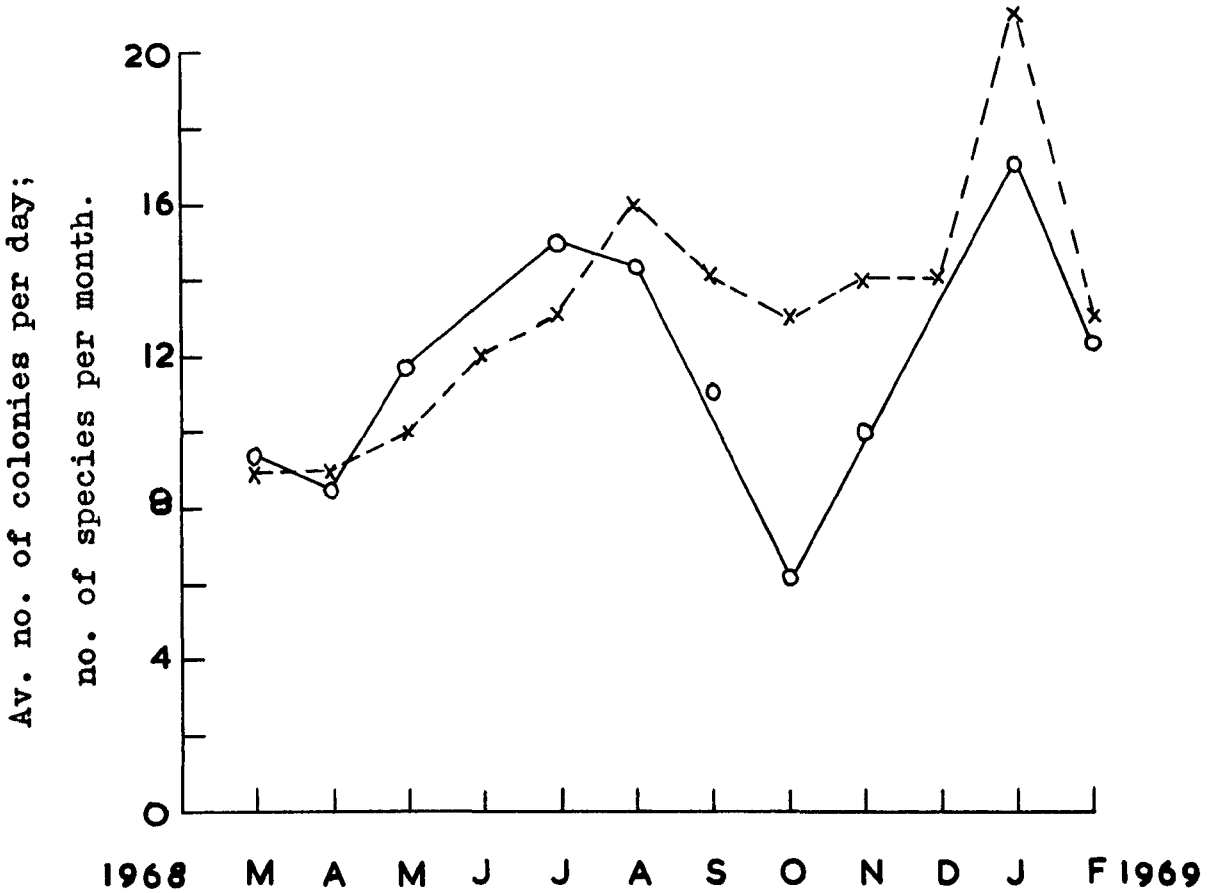


Figure 8

Average number of fungal colonies isolated from the air spora per day for each month of the sampling period (o——o), and the total number of species recorded per month (x--x).

number of thermophilous fungal species isolated during the spring was also low, but rose steadily through the summer months and reached a slight peak in August. The number fell slightly during autumn but proceeded to rise again during the early winter months and reached a definite maximum in January, followed by a gradual fall in February. The significant rise in species numbers over the January period was also reflected in the very high isolate counts recorded during this month. However, the low isolate counts obtained over the September to November period (dramatically low in September) was not equilibrated with a significant drop in the number of species isolated, although there was a slight reduction in species numbers during this period. There would appear to be, therefore, a certain amount of seasonal variation in the numbers of thermophilous fungal isolates and species in the atmosphere.

Over 2,000 isolates were obtained over the twelve month sampling period and by far the largest proportion of these isolates were represented by Aspergillus fumigatus (44.8% of the total isolations). Thermomyces lanuginosus (10.3%) and Mucor pusillus (9.1%) also formed a substantial proportion of the total isolations.

These three species were also the most frequently isolated species from the general habitats (see Chapter II) and also from the coal spoil tip profile, and this may be a direct result of their high sporulation ability and the adaptability of the spores to air dispersal. The dispersal and occurrence of some of these species in outdoor and indoor air was discussed by Austwick (1963) and A. fumigatus was reported as being prevalent in the air spora of the latter environments in particular.

Absidia corymbifera and Absidia ramosa (4.6%) were also substantially represented and were isolated particularly during the winter months. Other species which were isolated in significant amounts and may be termed common members of the thermophilous air spora include: Cephalosporium sp. 1 (3.3%), Penicillium piceum (2.8%), Dactylomyces crustaceus (2.3%), Aspergillus nidulans (1.8%) and Chrysosporium sp. (1.2%). Certain species occurred sporadically over the sampling period and constituted less than 1% of the total isolations but never less than 0.5%, these include: Paecilomyces varioti, Sporotrichum thermophile and Thermoidium sulphureum. The percentage frequency of isolation of these common species are presented in histogram form in Figure 9.

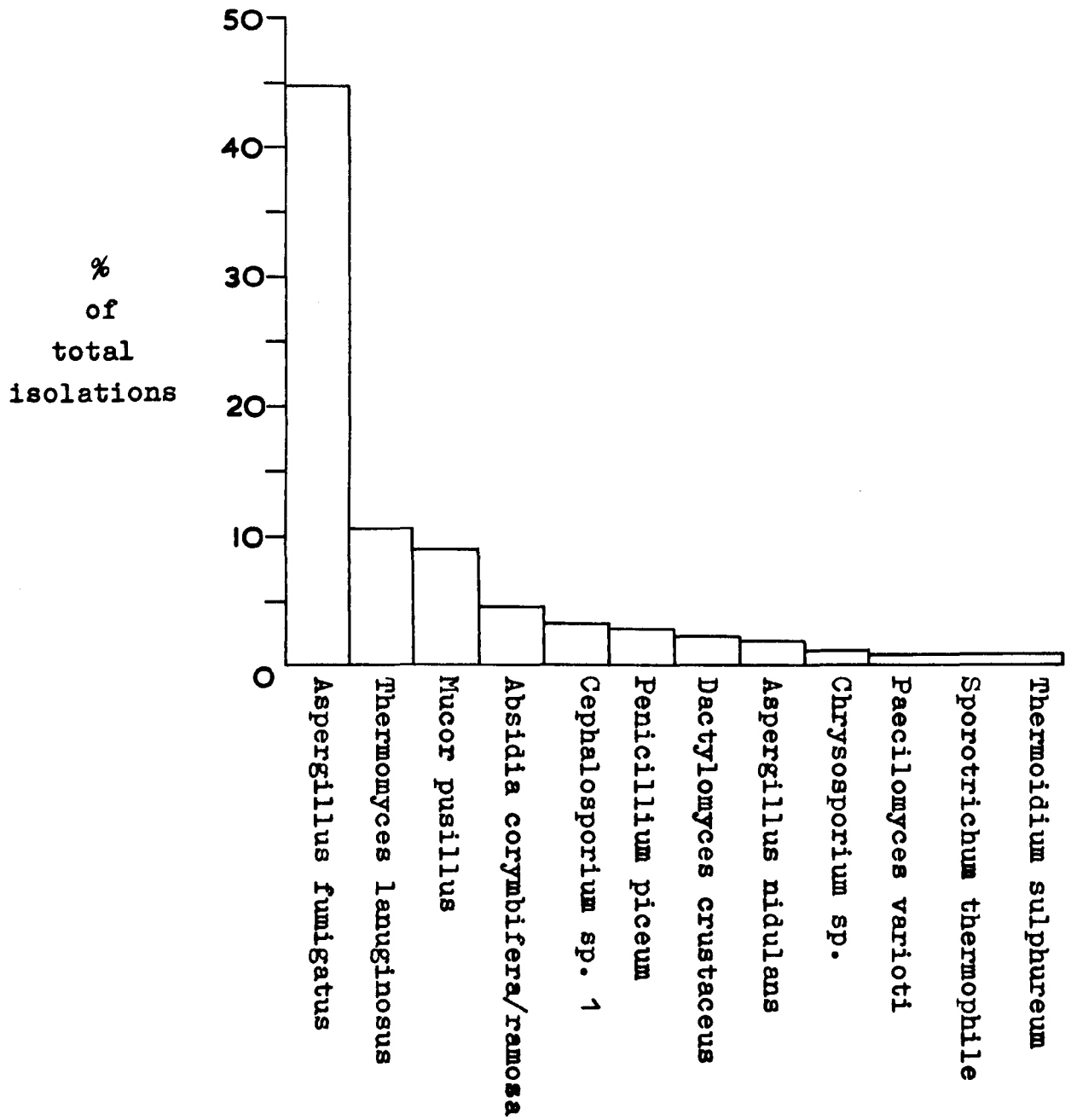


Figure 9

Percentage frequency of isolation of the commonest species recorded from the air spora.

All the above mentioned species have the ability to produce large numbers of relatively small asexual spores and hence this may account for their frequent appearance on exposed petri plates. It must be stated, however, that this method is basically non-quantitative, although it does give some measure of the kinds of viable spores present in the atmosphere and also of relative densities.

The remaining species were isolated in very small amounts, always representing less than 0.5% of the total isolations, and indeed some were only isolated on a single occasion. Their occurrence in the air spora would, therefore, appear to be limited. These species include: Allescheria terrestris, Aspergillus spp. 2 and 3, Chaetomium sp. 1, Humicola insolens, Penicillium sp. 1, Sporotrichum sp., Talaromyces duponti and Talaromyces emersonii. The following species were also rarely recorded but more specifically they occurred only on plates incubated at 45°C, viz: Monilia sp., Thielavia sepedonium and Trichoderma viride. This is probably near the maximum temperature for growth of these species and this may account for their limited occurrence, and in particular for their apparent absence from the other habitats studied.

Seasonal variation as applied to individual species was not in general, well defined, although species such as Humicola insolens, Talaromyces duponti and Thermoidium sulphureum were isolated almost exclusively during the winter months. Another species, Cephalosporium sp. 1, was isolated only during the summer months and occurred in substantial amounts comprising over 20% of the total isolations during these months. A similar situation was observed for Penicillium piceum which was isolated almost exclusively during the autumn and winter and for these months it formed a considerable proportion of the total isolations, approximately 5%. Mucor pusillus and Thermomyces lanuginosus occurred more frequently during the winter months than at any other time of the year, and this situation is very similar to that observed in the soil (see coal tip profile results).

General Discussion

Comparisons can be drawn between the results of the profile study and those of the air spora study. The dominant species in the air spora were also well represented in the upper layers of the coal spoil tip profile. Thus, the fungi present in these layers, and in the

topsoil especially, may be deposited there in substantial amounts from the atmosphere, and the species appearing on the soil incubation plates taken from these samples are probably derived directly from air-borne spores and not from active mycelia. Those species prevalent in the air spora markedly decreased in occurrence at the lower profile levels and only Aspergillus fumigatus commonly occurred. It is interesting to note that a number of the Ascomycetes, e.g. Chaetomium thermophile, Myriococcum albomyces and Thermoascus aurantiacus, were not recorded from the air spora but were isolated from the coal spoil tip profile, particularly from the lower layers, in fairly substantial amounts. Their apparent absence from the air spora may explain their scarce occurrence in the surface layers and particularly in the topsoil of the coal spoil tip profile. These species may be more frequently dispersed by animals (birds etc.).

Of the unusual species isolated from the coal spoil tips, only Penicillium sp. 1 was recorded from the air spora, and this only on one single occasion. It would appear, therefore, that they are poorly represented in the air spora, although their initial spread to the coal spoil tips and especially to the warm areas could have been by air.

The seasonal variation in the numbers of thermophilous fungal isolates and species recorded from the coal spoil tip (Leycett) is comparable with a similar variation in the air spora. Maxima were reached during the late summer and the winter months, with distinct minima occurring during autumn (September to October) and spring. The high number of isolates recorded in the summer months may be due to increased spore production during the periods of favourable environmental conditions, i.e. high temperature, although low soil moisture and organic matter content may restrict full development. During the winter months the high organic matter content of the soil, due to leaf-fall in the previous autumn, may encourage a second period of growth and subsequent spore production, facilitated by local areas of elevated temperature as a result of intense microbial activity. Nevertheless, it has not been established that soil temperatures are sufficiently elevated during the winter months to allow for the thermophilous type of growth. This can, however, be the most probable explanation of the significant increase in the thermophilous fungal populations (as reflected by soil and air spora studies) during these months. From the air and soil temperature

measurements (see Table 27) abnormally high temperatures were not recorded over the winter period.

CHAPTER IV

TEMPERATURE RELATIONSHIPS

i. Thermotolerance and Thermophily defined

According to Cochrane (1958) most fungi grow between the temperature limits of 10° and 40°C with an optimum somewhere around 25-35°C; such organisms are considered to be mesophiles. The number of fungal species able to grow above 45°C is small and they fall into two categories; thermotolerant and thermophilic. Cooney and Emerson (1964 p.6) fixed a definite standard temperature for the lower limit of growth of a true thermophile, at or above "room temperature" (approximately 20°C). Thus, an arbitary figure was chosen to fit a working definition of a thermophilic fungus, i.e. "one that has a minimum temperature for growth at or above 20°C". This definition is used by the present author to distinguish between a thermotolerant and a thermophilic fungus. A thermotolerant fungus is one that is able to grow at or above 45°C, usually having a maximum near 50°C, but is also able to grow below 20°C.

As stated earlier, previous workers have differed in their interpretations of thermophily. Craveri et al. (1964) and Craveri et al. (1967) regarded a true thermophile as being unable to grow below 25°C, and on this basis

relegated Mucor pusillus and Humicola insolens to the status of thermotolerants. However, in the present study they are classed as true thermophiles according to the working definition previously laid down. The term "thermophilous", as used by Apinis (1960, 1963a, 1963b, 1967) is used in this context to denote those fungi which are isolated at soil plate incubation temperatures of 45-50°C, and thus includes both thermotolerants and thermophiles. Apinis (1963a) divided thermophilous fungi into three groups depending upon their temperature relationships, viz:

a) microthermophilic species, with a temperature range not exceeding 40°C.

b) orthothermophilic species (true thermophiles).

c) psychrotolerant species, or the thermotolerant species as defined in the present text.

This, however, is not considered necessary in the present study due to the higher isolation temperatures employed, which exert a more selective effect on the variety of fungal species isolated, leading to the elimination of the species of group a).

ii. Determination of temperature relationships

Upon obtaining isolates which exhibited thermophilic growth tendencies it became necessary to ascertain whether

or not these organisms were thermotolerant or thermophilic, as previously defined. To accomplish this the optimum, maximum and minimum temperatures for growth had to be determined and for these determinations the diameter of growth method was used.

Brancato and Golding (1953) concluded that "the use of the diameter of the fungus colony as a measure of growth is considered sufficiently reliable for determining growth rates and for comparing the effect of environmental factors on fungus cultures on the same medium". In the present experiments the only medium used was potato dextrose agar because of its suitability as a non-specialised growth medium. Several other workers have agreed that, in studying the effect of temperature on the growth of a fungus colony, the diametric method may be used if all other variables are held constant. Crisan (1959) has shown that there is excellent correlation in determining optimum temperatures for growth when using different methods. The latter author compared colony diameter and mat weight growth of Aspergillus fumigatus and recorded essentially similar temperature relationships. Therefore, it was decided to use the colony diameter method because of its relative speed and simplicity in view of the larger number

of fungal species to be studied. It must be accepted, however, that the optimum temperature for growth may differ on various media because of the physiological requirements of different species, although the maximum and minimum growth temperatures are less likely to be affected.

Method

For each species four replicate plates were inoculated with standard inoculum (4 mm. plug) from actively growing colonies and incubated at the designated temperature. Periodically the colonies were measured to determine the increase in diametric or linear growth. Each colony was measured twice, the measurements being at right angles to each other in order to take into consideration possible variations of growth at different points on the circumference of the colony. The lag phase was restricted to a minimum by using material from actively growing colonies. At higher temperatures measurements were taken more frequently due to the increased growth rate, and maximum growth was recorded when the colony diameter was limited by the outer wall of the petri plate.

In a species with several strains, each strain was tested in order to determine whether there were differences in their temperature relationships.

Results

The following temperature scale was used during this study: 10, 12, 14, 16, 18, 20, 22, 25, 28, 30, 35, 37, 40, 42, 45, 48, 50, 52, 55, 57, 60 and 62°C. This wide temperature range was employed in order to ascertain the minimum, optimum and maximum temperatures for the growth of all the species isolated during the present study. In each incubator two thermometers were situated on the same level as the agar plates in order to determine the specific temperature immediately around the plates. Variations in the order of ±0.5°C occurred during incubation but in the water-jacket incubators especially, temperature gradients were neglible. Growth was measured over a ten day incubation period, and spore germination or traces of growth were detected by using a stereoscopic microscope.

The cardinal temperatures for growth are presented for each species and those species (or varieties) for which temperature-growth relationships have not previously been reported are discussed in greater detail and the temperature range of each plotted graphically.

Absidia corymbifera / Absidia ramosa

Cardinal temperatures °C.	min.	opt.	max.
A. corymbifera	14	40	50
A. ramosa	12	37	50

The temperature-growth relationships of these two species were found to be similar. A. corymbifera had slightly higher minimum and optimum temperatures for growth. However, both species grew at 50°C but failed to grow at 52°C. The thermotolerant nature of these two Absidia species has been known for a long time due to their occurrence in self-heating plant materials and their involvement in certain diseases of warm-blooded animals.

Mortierella sp.

Cardinal temperatures °C.	min.	opt.	max.
Strain 1	16	40-42	48
Strain 2	14	42	48

The temperature-growth relationships of this species are presented in Figure 10. The two strains which were examined were isolated from the warm areas of two different coal spoil tips. The minimum temperature for the growth

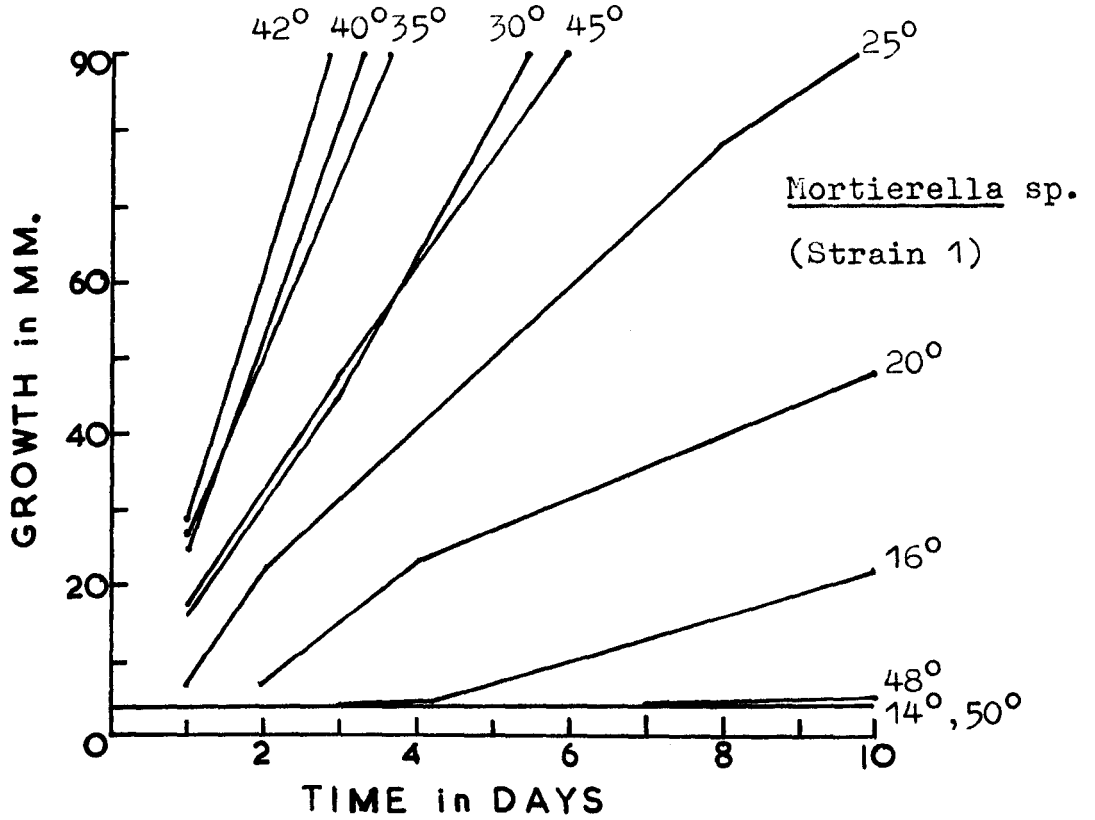
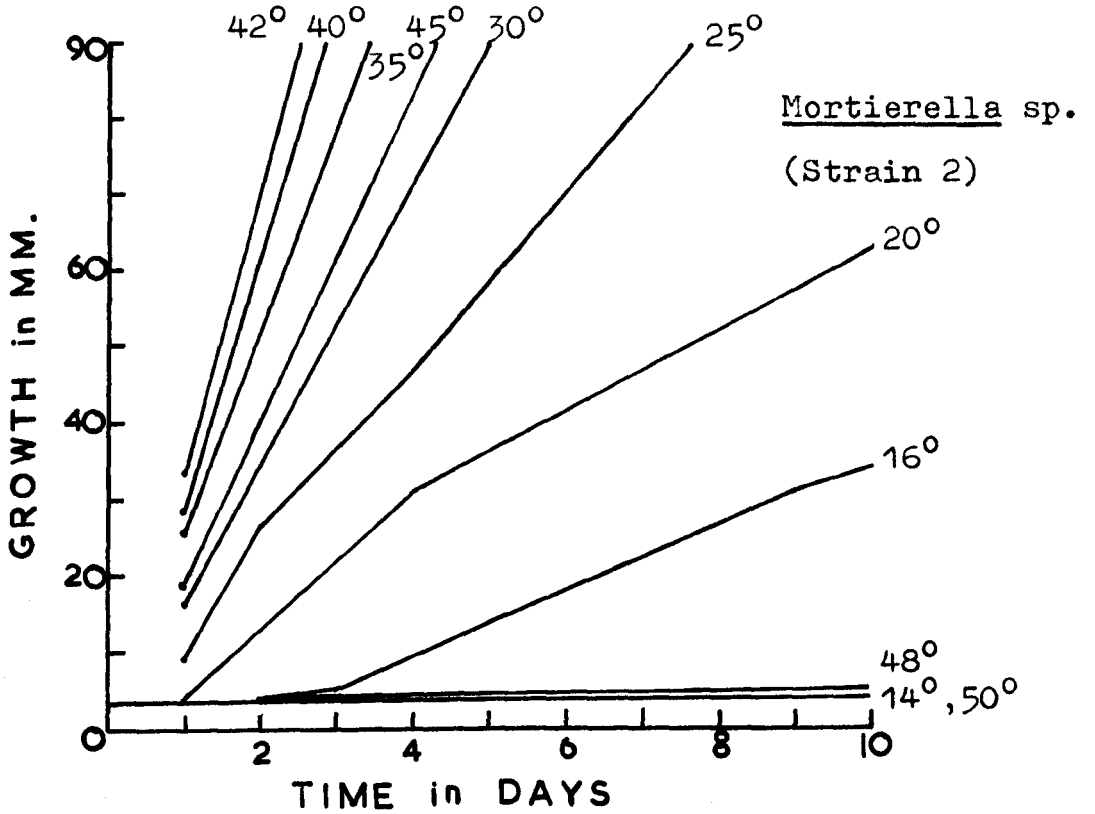


Fig. 10 Temperature-growth relationships



of these two strains was found to differ slightly, and only a trace of growth was present at these low temperatures. Growth below 25°C was extremely slow but above this temperature the growth rate markedly increased, and between 30°C and 40°C growth was extremely rapid. The optimum for both strains lay between 40°C and 42°C, although strain 2 had a slightly higher optimum and the growth rate of this strain was higher at 45°C than at 30°C, whereas the reverse applied to strain 1. Growth dramatically ceased above 45°C and only spore germination was detected at 48°C. This species would, therefore, be classified as thermotolerant.

Mucor pusillus complex

Cardinal temperatures °C.	min.	opt.	max.
Mucor miehei	22	45	57
Mucor pusillus	20	35-42	55
Mucor sp.	20	42	55

M. miehei grew extremely slowly below 30°C, whereas *M. pusillus* and *Mucor* sp. had lower minimum temperatures for growth and they grew quite vigourously at 25°C and 30°C. Optimum growth for *M. miehei* occurred at 45°C and maximum growth at 57°C. However, these temperatures were

lower for M. pusillus and Mucor sp., and these species would appear to be less thermophilic than M. miehei. These species can all be classified as thermophiles as growth below 20°C was not detected. Nevertheless, the low minimum temperatures for growth shows weak thermophilic tendencies.

Rhizopus sp. 1

Cardinal temperatures °C.	min.	opt.	max.
	25	45-52	60

The temperature-growth relationships of two strains of this species are presented in Figure 11. A trace of growth was present at 25°C, but no growth was detected for the first six days, and above 30°C there was a dramatic increase in the growth rate. Growth was rapid between 35°C and 55°C with the optimum rate occurring over a wide temperature range, i.e. 45-52°C. At 57°C growth was still relatively rapid in strain 1 but in strain 2 growth slackened and fell below the amount recorded at 30°C. Appreciable growth occurred at 60°C and this species would appear to be strongly thermophilic, certainly the most thermophilic Phycomycete so far recorded.

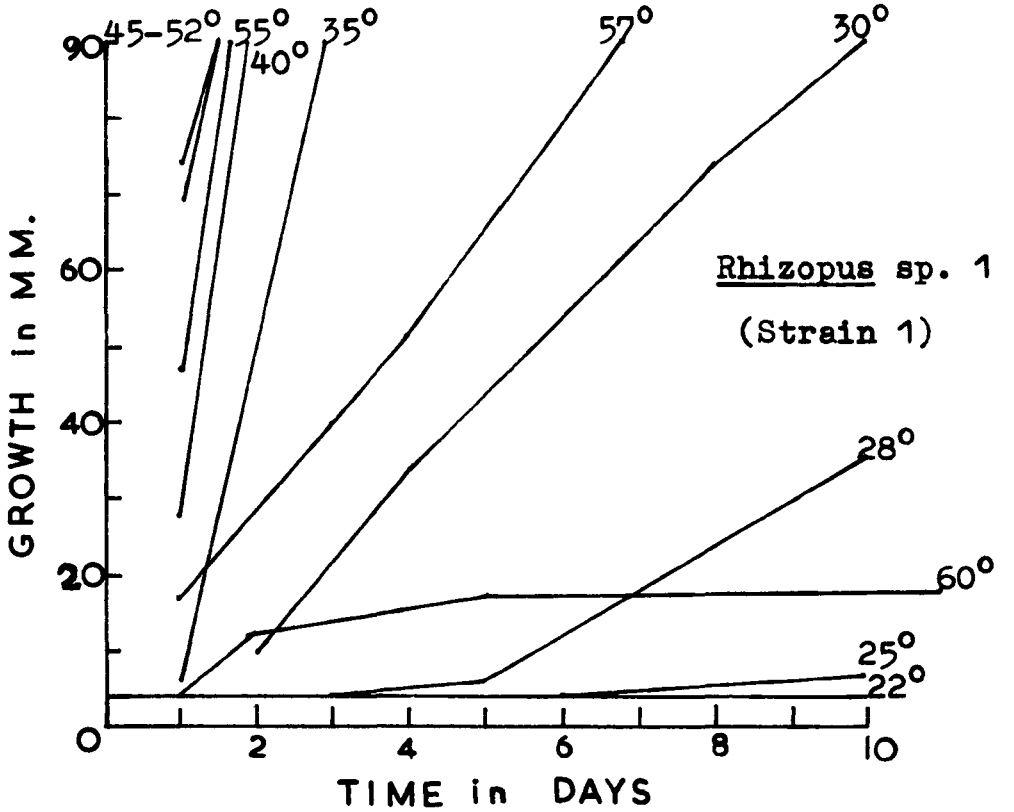
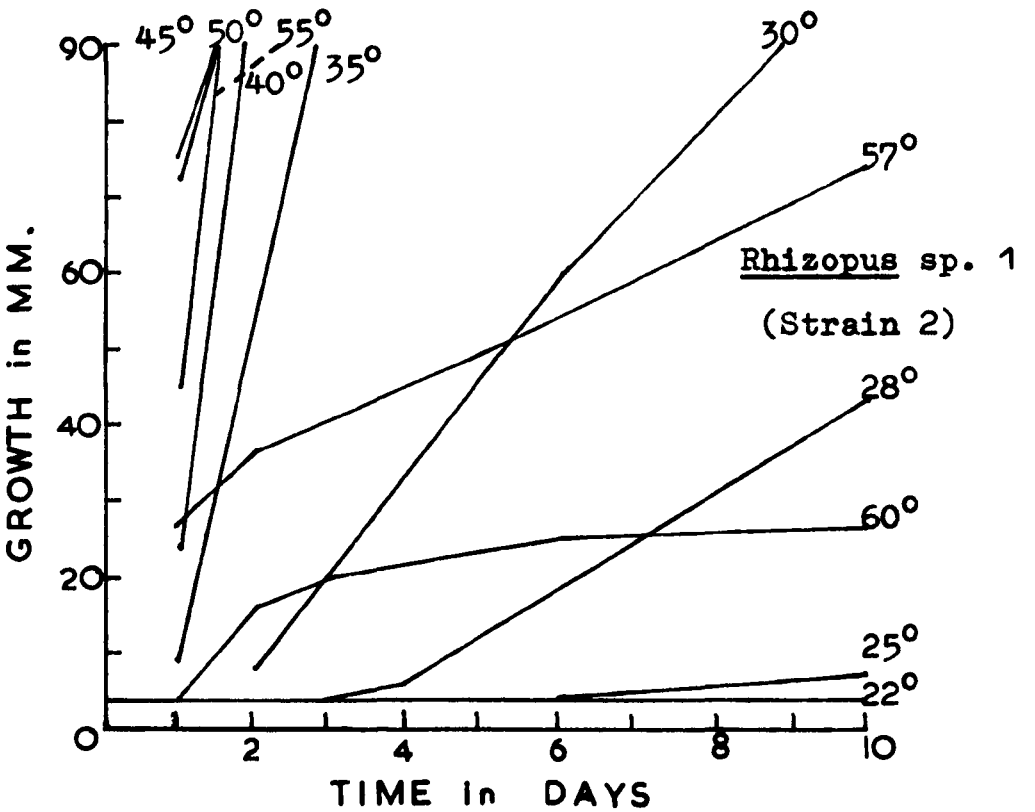


Figure 11 **Temperature-growth relationships**



Rhizopus sp. 2

Cardinal temperatures °C.	min.	opt.	max.
	12	40	50

The temperature-growth relationships of this species are presented in Figure 12a). No growth was present at 10°C but at 12°C and 14°C the growth rate after an initial lag phase was fairly rapid. Between 20°C and 45°C growth was extremely rapid with the optimum occurring at about 40°C, and the optimum range at 30-45°C. Above 45°C there was a marked decline in the growth rate and growth was slight at 50°C. Growth was absent at 52°C. This species can be regarded as a weak thermotolerant due to its inability to grow above 50°C and its ability to grow well at and below 20°C.

Rhizopus sp. 3

Cardinal temperatures °C.	min.	opt.	max.
	10	42	55

This was an extremely fast-growing species over a wide temperature range (see Figure 12b). A trace of growth was recorded at 10°C; at 20°C and above the growth rate rapidly increased and growth was very fast between 30°C

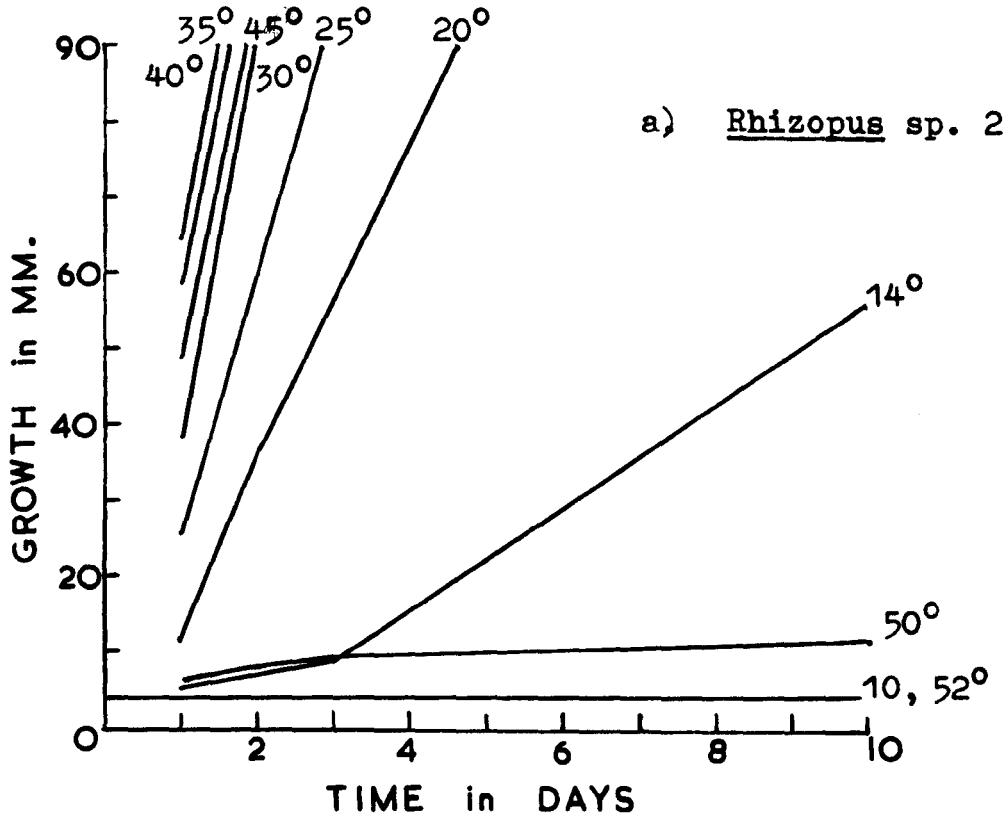
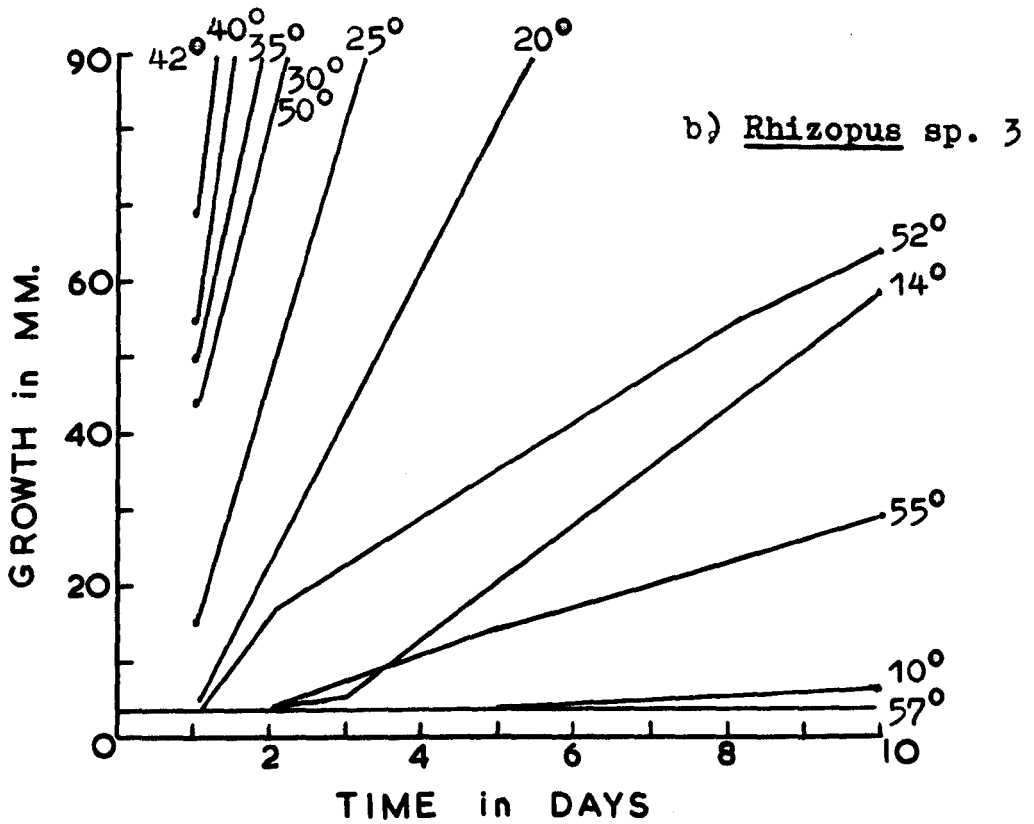


Figure 12 Temperature-growth relationships



and 50°C. A clear optimum was evident at 42°C, above 50°C growth diminished but was still significant at 52°C and 55°C. No growth was detected at 57°C. This is a thermotolerant species but one having marked thermophilic tendencies due to its ability to grow reasonably well above 50°C.

Ascomycetes

Allescheria terrestris

Cardinal temperatures °C.	min.	opt.	max.
Strain 1	22	45	55
Strain 2	22	42-45	55

Two strains were examined and at 22°C both strains showed traces of growth after the ten day incubation period. In strain 1 a well defined optimum occurred at 45°C but an optimum temperature range of 42-45°C was recorded for strain 2. Above 50°C growth was greatly reduced and at 57°C no growth was detected.

Bergman and Nilsson (1966, 1967) reported similar temperature-growth relationships for A. terrestris, i.e. optimum at 45°C and maximum at 55°C. At the latter temperature only a trace of growth was recorded after

seven days incubation. This species can be regarded as a true thermophile but not strongly thermophilic.

Chaetomium thermophile

Cardinal temperatures °C.	min.	opt.	max.
var. coprophile	28	45-50	60
var. dissitum	28	45-48	60

The temperature-growth relationships of these two varieties were found to be very similar. Growth was extremely slow at 28°C and up to 35°C, but between 40°C and 55°C the growth rate was extremely rapid, with the optimum lying between 45°C and 50°C. Above 55°C growth rapidly slowed down and at 60°C only a trace of growth occurred. This species would appear, therefore, to have very strong thermophilic tendencies due to the high minimum and maximum temperatures for growth.

Cooney and Emerson (1964, p.69) list similar temperature-growth relationships for C. thermophile and its varieties, although they recorded no growth at 60°C. Other workers (Craveri et al. 1967, Chang 1967 and Maheshwari 1968) also recorded the failure of this species to grow at 60°C. Cooney and Emerson noted that var. coprophile grew much faster at 50-55°C than var. dissitum

and this was especially noticeable during the present studies.

Chaetomium sp. 1

Cardinal temperatures °C.	min.	opt.	max.
	14	40	52

A trace of growth occurred at 14°C and up to 20°C the growth rate was slow. Above 20°C there was a marked increase in the growth rate and rapid growth occurred between 30°C and 45°C, the growth rate being very similar at these two temperature limits (see Figure 13a). Optimum growth occurred at about 40°C. Above 45°C the growth rate gradually declined and slight growth was present at 52°C. The ability of Chaetomium sp. 1 to grow at temperatures below 20°C and the marked reduction of growth at temperatures above 45°C determines this species as thermotolerant.

Chaetomium sp. 2

Cardinal temperatures °C.	min.	opt.	max.
	16	40	52

The temperature-growth relationships of this species are presented in Figure 13b). At 16°C and 18°C growth was

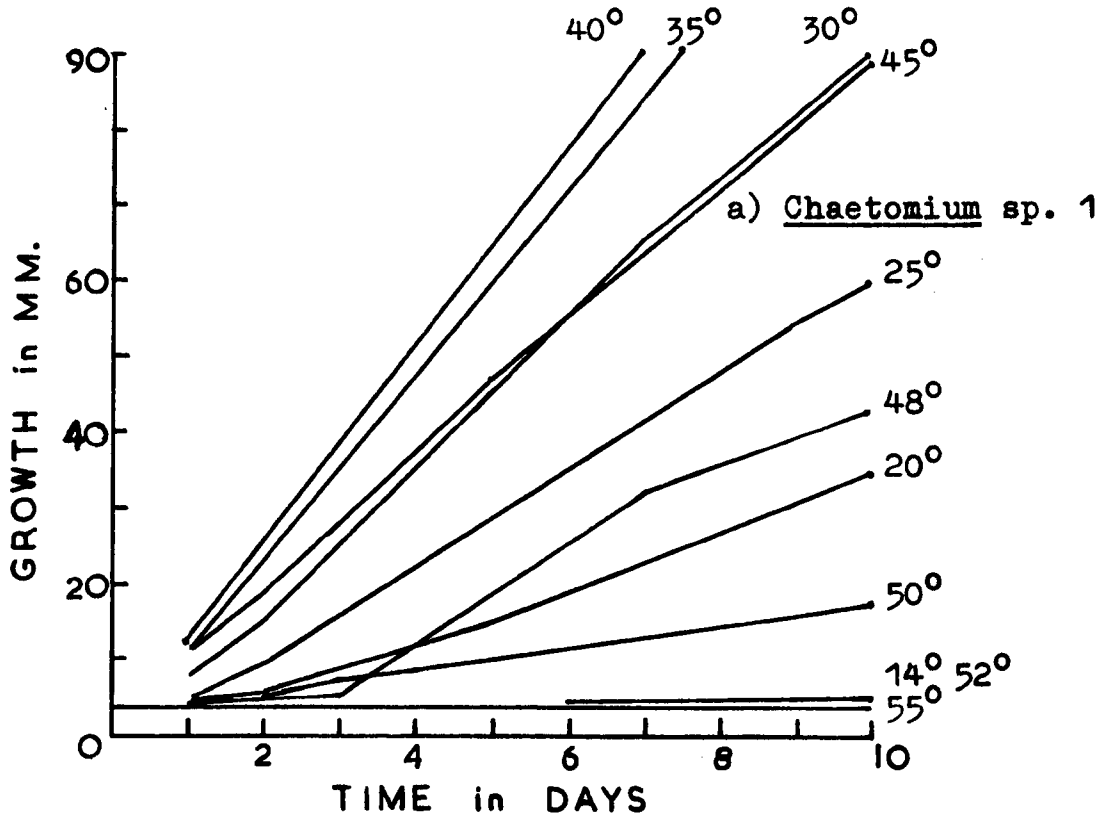
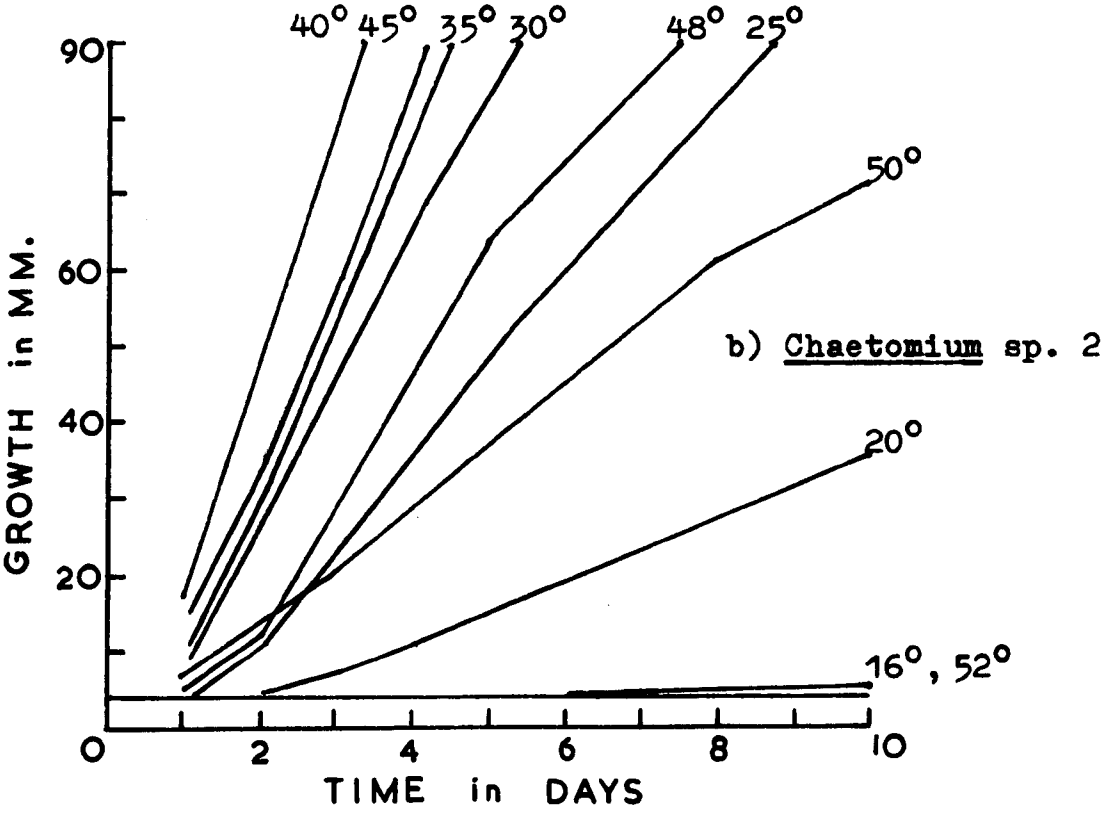


Figure 13 **Temperature-growth relationships**



slight, but significant at 20°C. Above this temperature there was a marked increase in the growth rate and between 25°C and 48°C a rapid growth rate was maintained, with the optimum occurring at 40°C. Growth at 45°C was still faster than at 37°C and 35°C. At 50°C the growth rate fell slightly and above this temperature there was a dramatic decrease in the growth rate and at 52°C only a slight growth occurred. This species would appear to have slightly stronger thermophilic tendencies than Chaetomium sp. 1, due to its higher minimum temperatures for growth and its relatively rapid growth rate at 45°C and 50°C. However, both these species were markedly affected by temperatures above 50°C and failed to grow at 55°C.

Dactylomyces crustaceus

Cardinal temperatures °C.	min.	opt.	max.
	20	37	55

The temperature-growth relationships of D. crustaceus are presented in Figure 14a). Slight growth was recorded at 20°C, whilst at 30°C growth was greatly increased. Rapid growth occurred between 35°C and 40°C with the optimum growth rate at 37°C. Above 45°C growth gradually

decreased and little growth was recorded at 55°C. No growth was present at 57°C, and because of the failure of this species to grow at 18°C it can be regarded as a true thermophile from the definition by Cooney and Emerson. However, the low minimum and optimum temperatures for growth of D. crustaceus show weak thermophilic properties.

Dactylomyces thermophilus

Cardinal temperatures °C.	min.	opt.	max.
	18	42	55

A trace of growth was present at 18°C, but at 20°C growth was significantly increased. Rapid growth was recorded over a wide temperature range, i.e. 30-50°C, with the optimum growth rate occurring at 42°C. Above 50°C growth decreased markedly and very slight growth was recorded at 55°C. Because of the ability of D. thermophilus to grow below 20°C this species is classed as a thermotolerant, but nevertheless its relatively high optimum and maximum temperatures for growth are similar to those of a number of true thermophiles. From a comparison of the temperature-growth relationships of D. crustaceus and D. thermophilus (see Figure 14b) it can be seen that the latter species had a higher growth rate at 45°C and 50°C than the former

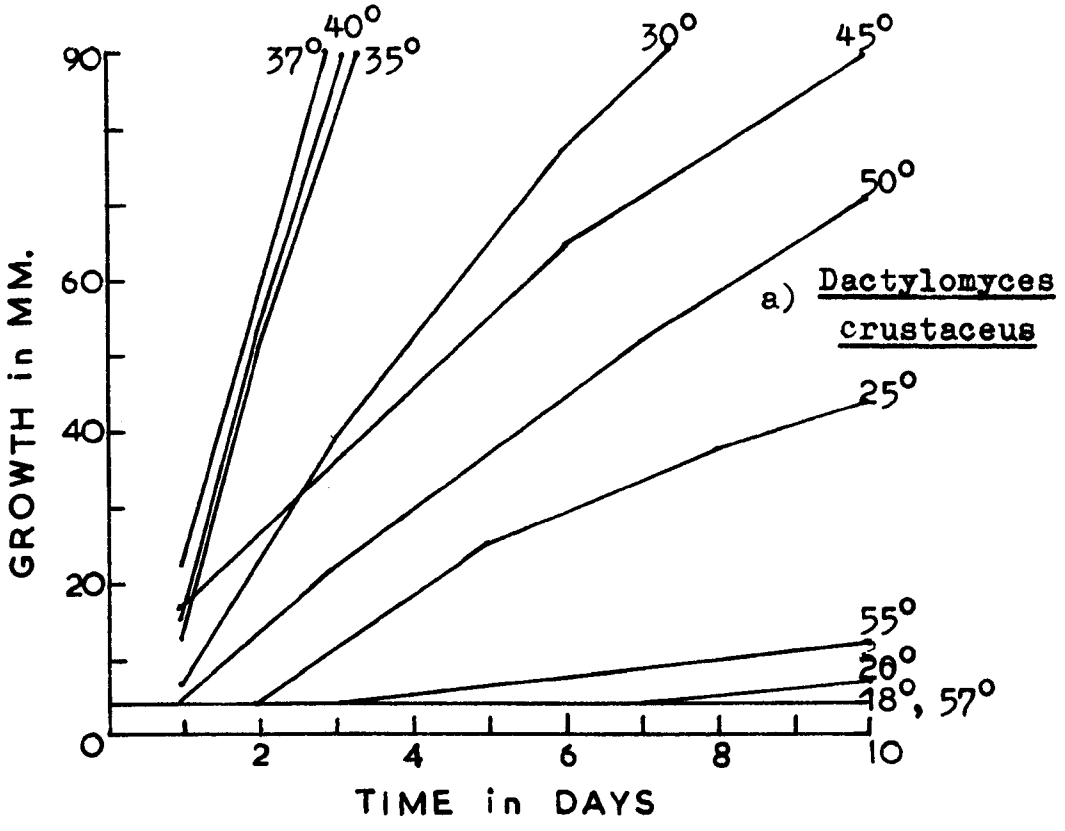
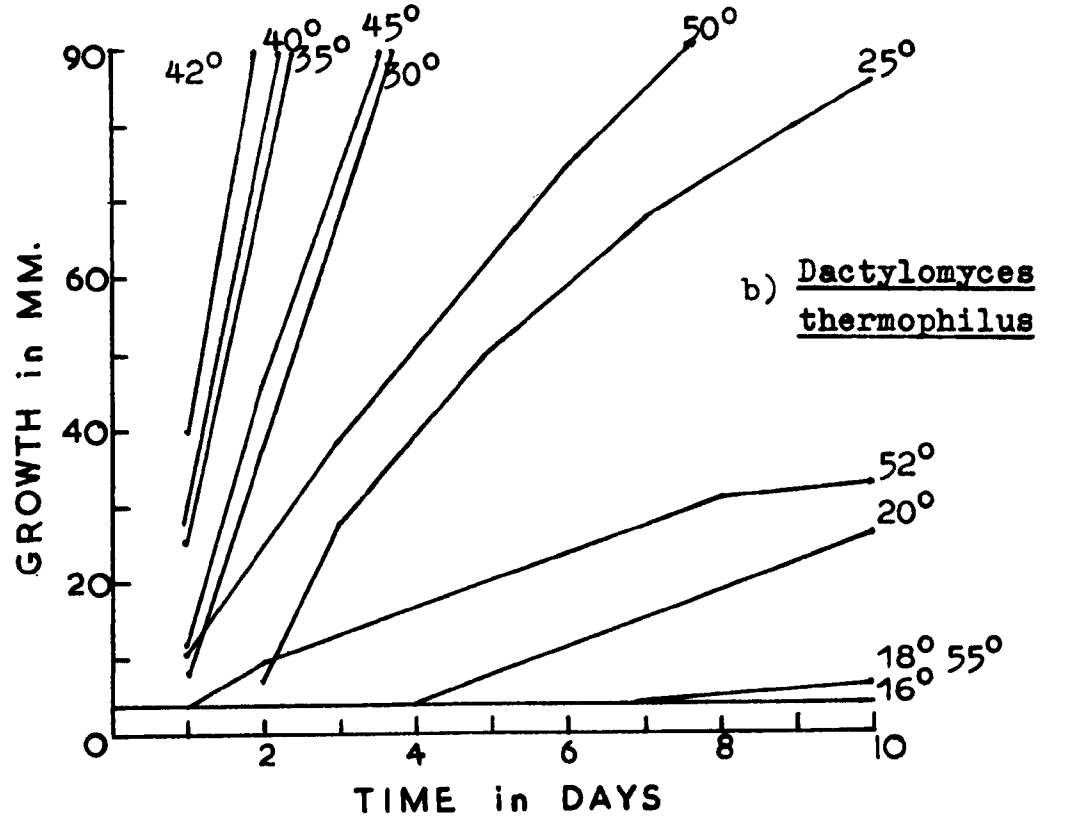


Figure 14 **Temperature-growth relationships**



species, and it would appear at these temperatures to have stronger thermophilic tendencies than D. crustaceus. Hence, the definition between thermotolerant and thermophilic species becomes anomalous and the full implications will be discussed later.

Myriococcum albomyces

Cardinal temperatures°C	min.	opt.	max.
	25	45	55

At 25°C slight growth was recorded for M. albomyces but below this temperature growth was not detected. Growth was rapid between 40°C and 50°C and a clear optimum was present at 45°C. Above 50°C growth was significantly reduced and at 57°C growth was not apparent after the ten day incubation period.

These results differ in several respects from those reported by Cooney and Emerson (1964, p.61). The latter authors quoted minimum at 27°C, optimum between 37°C and 42°C, and maximum at 57°C. There would, therefore, appear to be differences in the temperature-growth relationships of strains of M. albomyces, and this may ultimately be connected with environmental conditions of the habitat from which the strains were originally isolated.

However, M. albomyces can be classed as a true thermophile due to its high minimum temperature for growth.

Talaromyces duponti

Cardinal temperatures °C.	min.	opt.	max.
Strain 1	30	50	60
Strain 2	28	50	60

Two strains of T. duponti were examined and slight differences in their temperature-growth relationships were found. A trace of growth at 28°C was recorded for strain 2 but for strain 1 growth did not occur until 30°C. At 30-37°C growth was relatively sparse and strain 2 had the fastest growth rate at these lower temperatures. Above 40°C the growth rate of the two strains increased markedly, especially strain 1, which was by far the most vigorous grower at the higher temperatures. Optimum growth for both strains was well defined at 50°C and only above 55°C was the growth rate significantly reduced. Due to the high cardinal temperatures for growth T. duponti would appear to be a species with very strong thermophilic properties.

Similar cardinal temperatures have been shown for T. duponti by Cooney and Emerson (1964) and growth of this

species at 60°C has been reported by Griffon and Maublanc (1911) and Maheshwari (1968).

Talaromyces emersonii

Cardinal temperatures °C.	min.	opt.	max.
T. emersonii	25	45	60
T. emersonii var. 2	28	45	60

The temperature-growth relationships for T. emersonii and its varieties are presented in Figure 15a). Variety 1 was very similar to the normal strain of T. emersonii in its temperature requirements and hence the results are not presented.

T. emersonii grew slowly at 25°C but above this temperature the growth rate rapidly increased and between 40°C and 57°C a rapid growth rate was maintained, with the optimum occurring at about 45°C. Above 57°C growth rapidly decreased but was still significant at 60°C. T. emersonii var. 2 (see Figure 15b) failed to grow at 25°C and very little growth was recorded at 28°C and 30°C. A high growth rate occurred between 40°C and 57°C, as above, but a clear optimum growth rate was apparent at 45°C. At the latter temperature growth was nearly twice as fast as at any other temperature in this range.

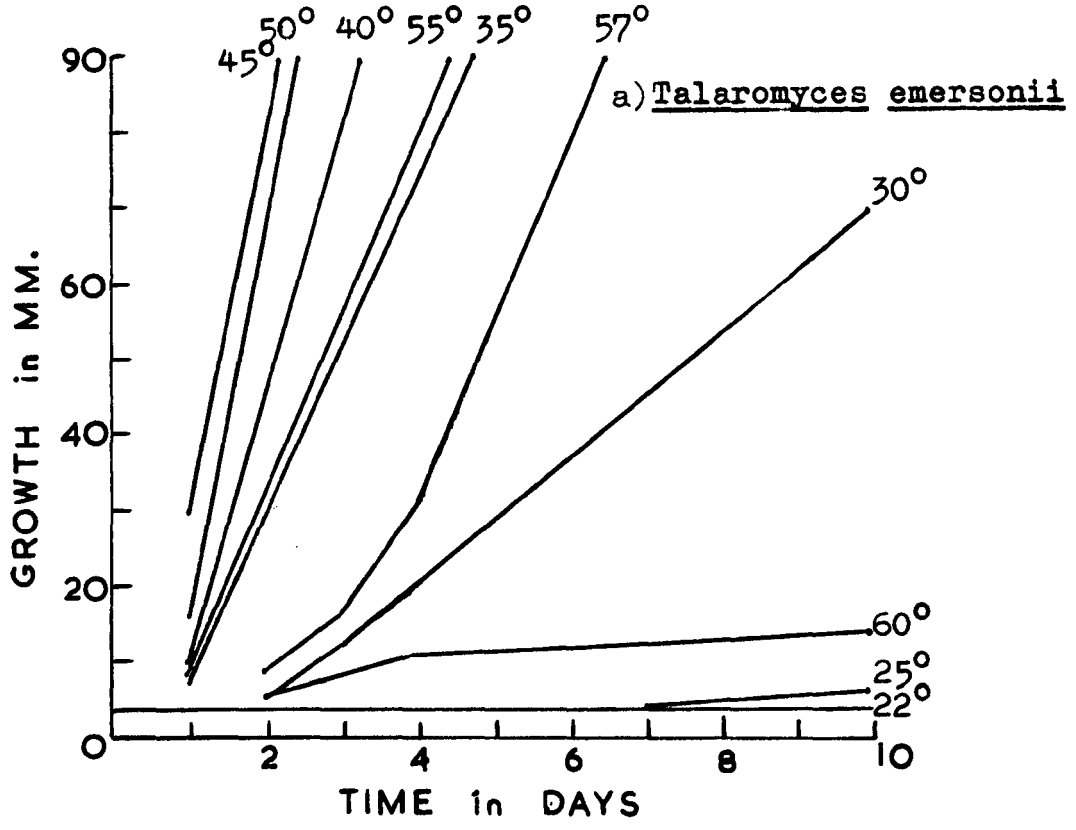
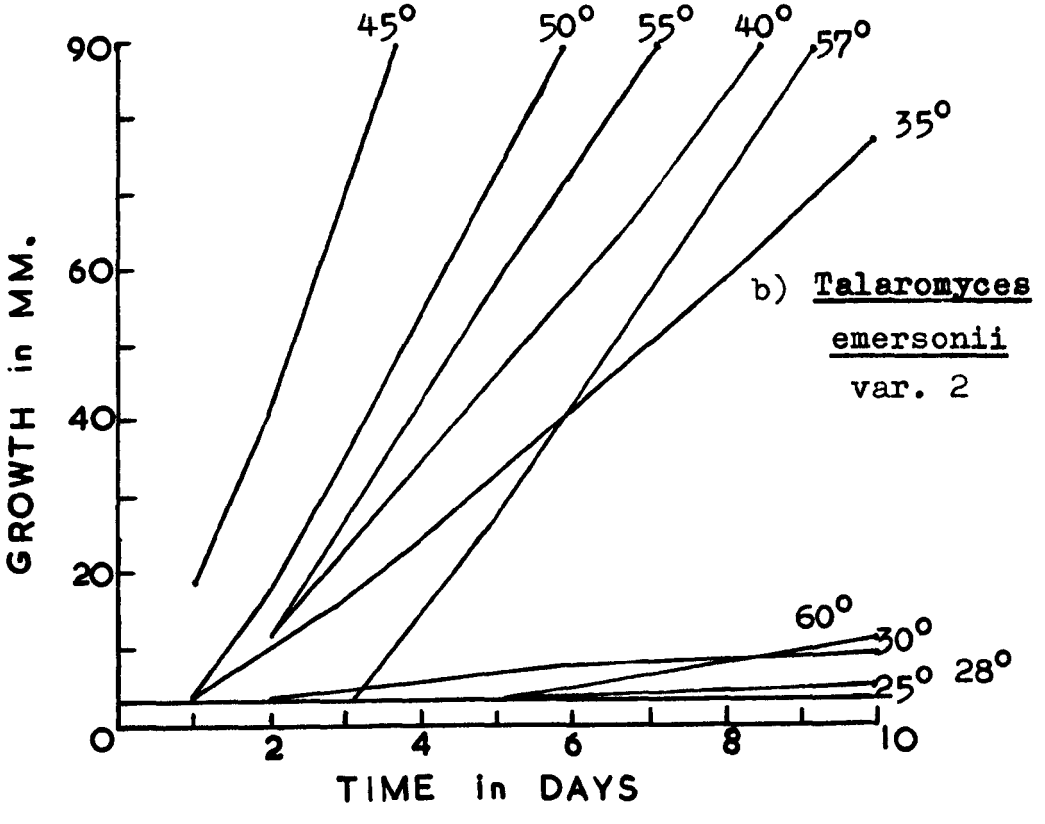


Figure 15 **Temperature-growth relationships**



Above 57°C the growth rate was markedly reduced and little was recorded at 60°C.

Stolk (1965) reported no growth at 25°C for the strains of T. emersonii which she examined. At 30°C slight development was reported and optimal development occurred at 40-45°C. Limited growth was present at 55°C and growth was absent at 60°C. The strains examined in the present study would appear to have a much wider temperature range for growth, i.e. 25-60°C, and to be more strongly thermophilic at elevated temperatures, i.e. faster rate of growth at 55°C and 57°C than the strains investigated by Stolk. Bergmann and Nilsson (1966) also reported growth of T. emersonii at 60°C. Nevertheless T. emersonii is a true thermophile with strong thermophilic properties.

Talaromyces sp.

Cardinal temperatures °C.	min.	opt.	max.
	18	42	55

The temperature-growth relationships of this species are presented in Figure 16a). A trace of growth was recorded at 18°C and 20°C, although at 18°C growth did not occur until the eighth day of incubation. At 25°C the growth

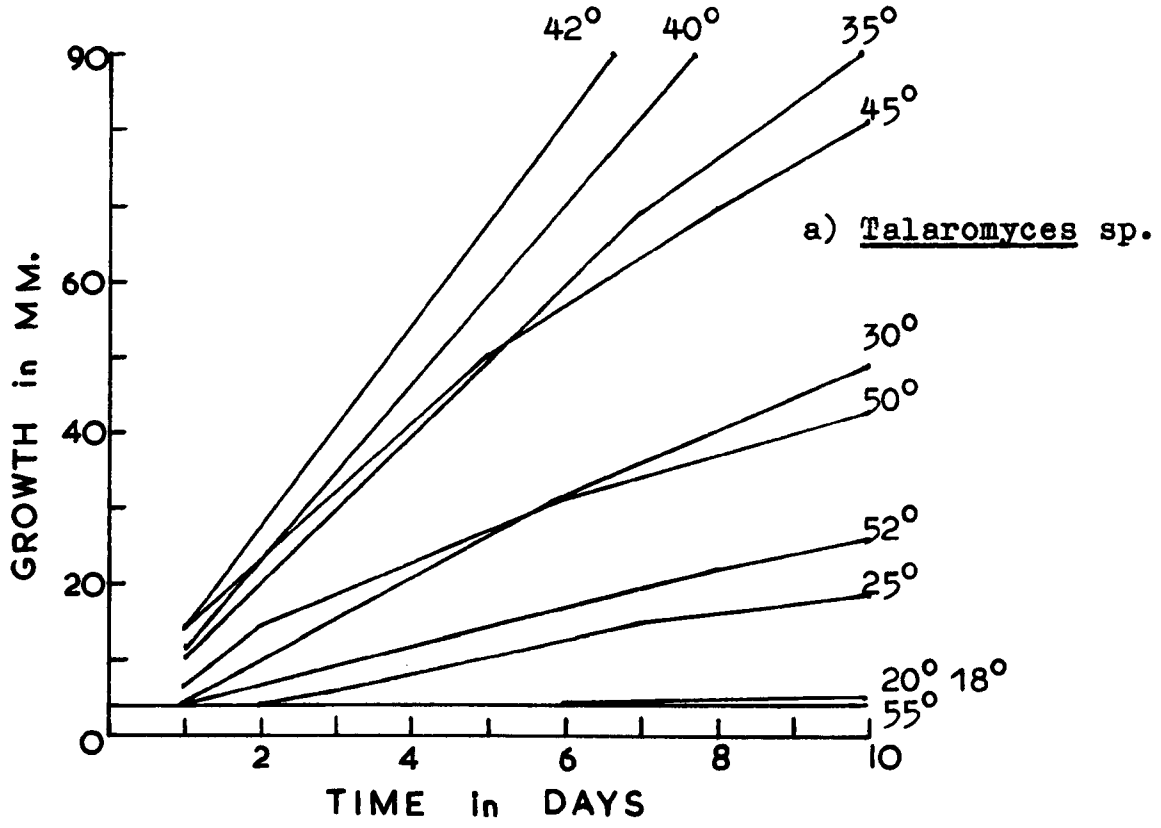
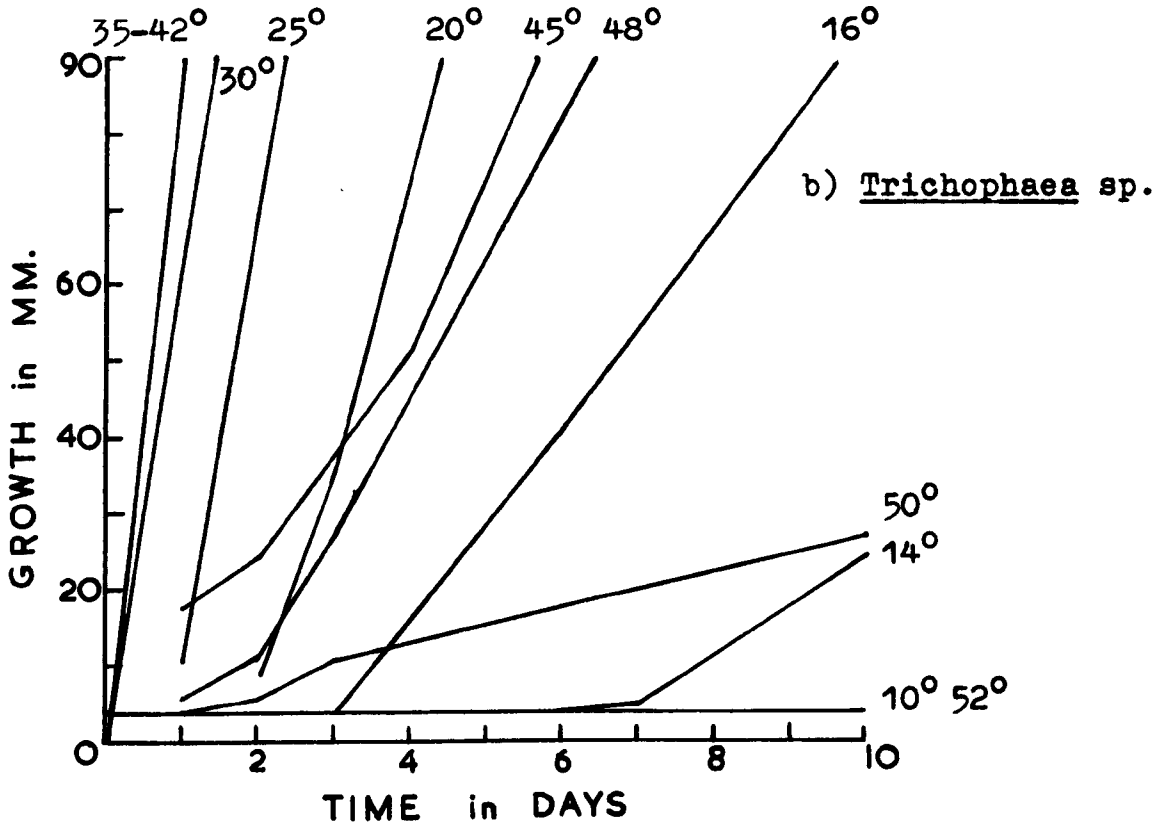


Fig. 16 Temperature-growth relationships



rate was still slow but above 30°C the growth rate rapidly increased. Between 35°C and 45°C a high growth rate occurred and a well defined optimum was present at 42°C. Above 45°C growth decreased rapidly and was absent at 55°C being represented only by spore-germination.

Thermoascus aurantiacus

Cardinal temperatures °C.	min.	opt.	max.
Strain 1	30	45	62
Strain 2	35	45	62

The temperature-growth relationships of the two strains of T. aurantiacus are presented in Figure 17. Strain 1 was isolated from normal areas (pine woods) and strain 2 was isolated from warm areas (sewage). The former strain had a lower minimum temperature for growth (30°C) than the latter strain (35°C) and it also possessed a higher growth rate at 37°C and 40°C. Strain 2, on the other hand, had a higher growth rate at elevated temperatures and grew well at 57°C and 60°C. However, in strain 1 the growth rate markedly decreased above 57°C although at 60°C the amount of growth produced was still significant. A trace of growth at 62°C was observed for strain 1 but growth was still well represented for strain 2 at this temperature.

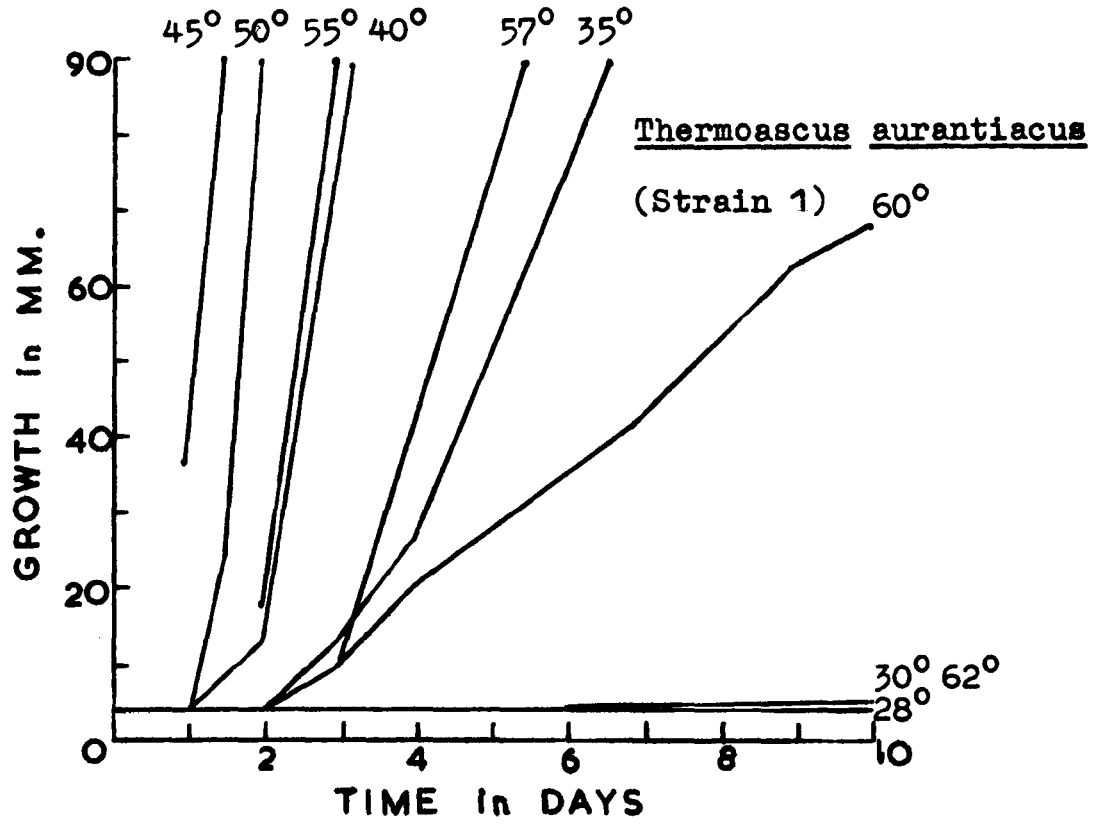
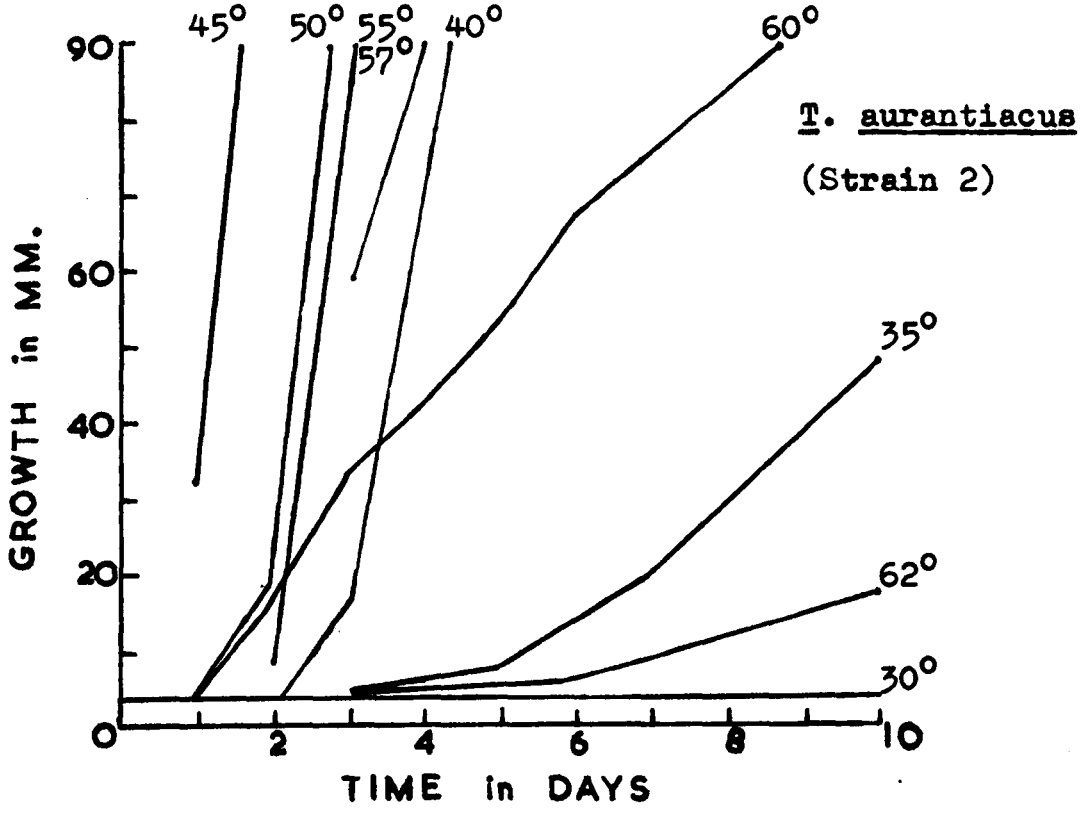


Figure 17 **Temperature-growth relationships**



At the higher temperatures the growth patterns were occasionally erratic. For example, at certain temperatures growth was apparently absent for the first few days of incubation when a sudden flare-up occurred and maximum growth was recorded in less than twenty-four hours, e.g. strain 2 at 55°C and 57°C. It can be concluded that strain differences may occur in the temperature-growth relationships of T. aurantiacus and that these may be connected with conditions present in the original habitat of any one particular strain. T. aurantiacus would appear to be one of the most strongly thermophilic species so far recorded.

Apinis (1963a) reported that this species grew after four days incubation at 57-58°C, although Craveri et al. (1967) recorded no growth above 50°C after an incubation period of nine days. This anomaly can be explained if the latter workers were basing their description of T. aurantiacus on that given by Cooney and Emerson (1964, p.39). The species that they described has been shown by Stolk (1965) and Apinis (1967) to be synonymous with Dactylomyces crustaceus. This is also obvious from the temperature-growth relationships which they list for T. aurantiacus, i.e. minimum 20-25°C, optimum 40-45°C

and maximum 55°C. These cardinal temperatures for growth are in excellent agreement with those reported for D. crustaceus (see Figure 14a).

Bergman and Nilsson (1966) examined strains of T. aurantiacus from wood chip piles and found similar temperature-growth relationships to those obtained during the present study, i.e. 35-60°C.

Trichophaea sp.

Cardinal temperatures °C.	min.	opt.	max.
	12	35-42	50

The temperature-growth relationships of this species are presented in Figure 16b). Growth was very slight at 12°C but above 16°C the growth rate rapidly escalated and a wide optimum temperature range occurred between 35°C and 42°C. Between 25°C and 48°C a rapid growth rate was maintained but above 48°C growth was greatly affected and at 52°C no growth was detected. This species has, therefore an extremely rapid growth rate over a wide temperature range. However, at the limits of this fast growing range, rapid metabolic slowing-down occurs over a few degrees temperature change, i.e. 16-14°C, 48-50°C, resulting in a dramatic decrease in development.

El-Abyad and Webster (1968) studied the physiological properties of pyrophilous discomycetes in relation to the physio-chemical and biological properties of burnt ground. The temperature-growth relationships of Trichophaea abundans, closely related to the present species, were investigated. They were found, however, not to differ significantly from those of normal soil fungi, i.e. no growth above 37°C, thereby showing no thermophilic tendencies. This can be considered unusual in view of the high temperatures attained by soil after burning, which may persist for long periods. The extremely fast growth rate of T. abundans was suggested as being advantageous in the exploitation of new habitats, i.e. burnt soil.

The colonisation by Trichophaea sp. of coal spoil tips, and the warm areas in particular, may be due to the ability of this species to tolerate high temperatures, as well as the other environmental extremes, e.g. high pH and sulphur content, low soil moisture-holding capacity. Indeed, the extremely rapid growth rate of this species over a very wide range of temperatures might, as proposed above, be uppermost in establishing Trichophaea sp. on coal spoil tips.

Fungi Imperfecti

Aspergillus fumigatus

Cardinal temperatures °C.	min.	opt.	max.
Green strain	12	40	55
Orange strain	12	42	55

The temperature-growth relationships of these two strains were found to be similar. A trace of growth was detected at 12°C and rapid growth occurred between 30°C and 50°C. Optimal development was slightly different for the two strains; the normal green strain had its optimum at 40°C while the orange strain had a higher optimum, at 42°C. Growth was still evident at 55°C but absent at 57°C.

Crisan (1959) quoted higher minimum and lower maximum temperatures for growth of the strains which he examined. Nevertheless, A. fumigatus would appear to have a very wide temperature range for growth, with the ability to grow well at low and high temperatures and this may partly explain its ubiquitous distribution.

Aspergillus nidulans / Aspergillus terreus

Cardinal temperatures °C.	min.	opt.	max.
A. nidulans	10	35-37	50
A. terreus	10	35	50

The temperature-growth relationships of these two species were found to be very similar. They both had a wide temperature range for growth with rapid growth between 30°C and 40°C. Above 45°C growth was markedly decreased and slight growth occurred at 50°C. These species are obviously not as thermotolerant as A. fumigatus and grow less vigorously at the higher temperatures.

Aspergillus sp. 1 to 5

Several of these species were found to be very difficult to measure accurately, due to their rapid spread on agar plates by numerous small colonies developing from individual or groups of spores. Therefore, only minimum and maximum temperatures for growth could be accurately tabulated but where it was possible to define the optimum temperature for growth then these results are presented.

Cardinal temperatures °C.	min.	opt.	max
Aspergillus sp. 1	12	35-37	48
" sp. 2	12	-	50
" sp. 3	10	-	50
" sp. 4	12	-	52
" sp. 5	14	42	55

Aspergillus sp. 1 to 5 all had wide temperature ranges for

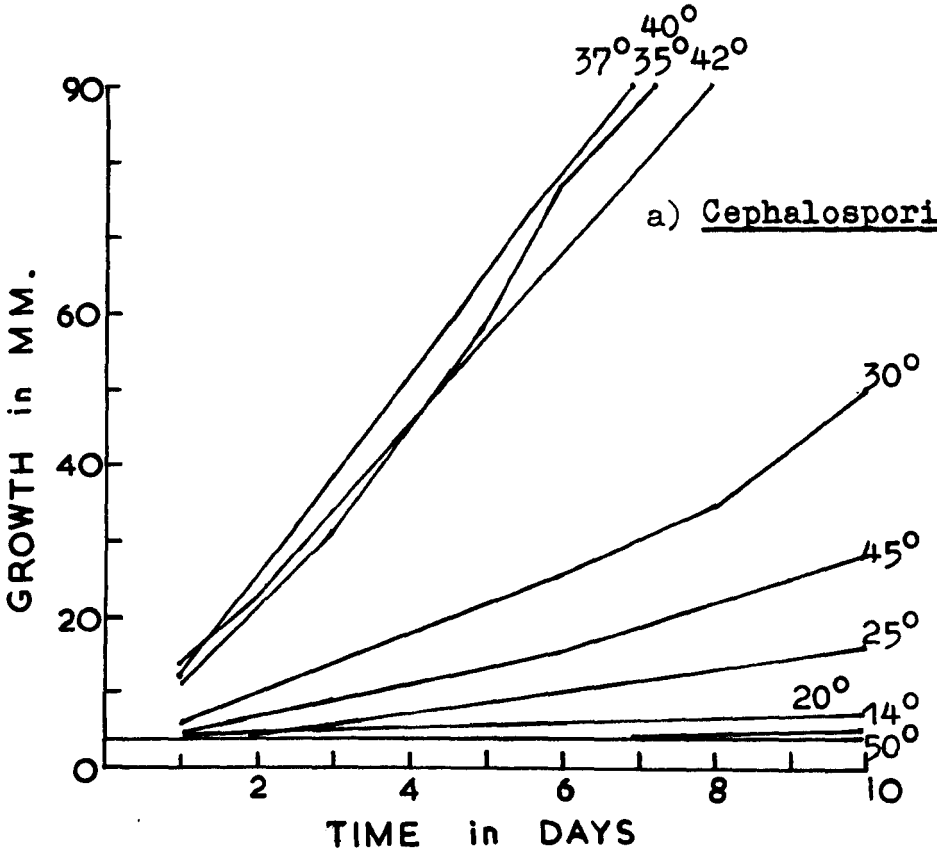
growth, similar to the other species of Aspergillus investigated (see above). Growth was rapid for all the species between 30°C and 40°C and for several of them growth was rapid up to 48°C. At or above this temperature the growth rate was markedly affected, and only one species (Aspergillus sp. 5, see Figure 20b) was able to grow at 55°C.

The prevalence of Aspergillus species in the warmer countries of the world suggests that this group of fungi consistently have wide temperature ranges for growth, with high optimum temperatures, i.e. above 30°C.

Cephalosporium sp. 1

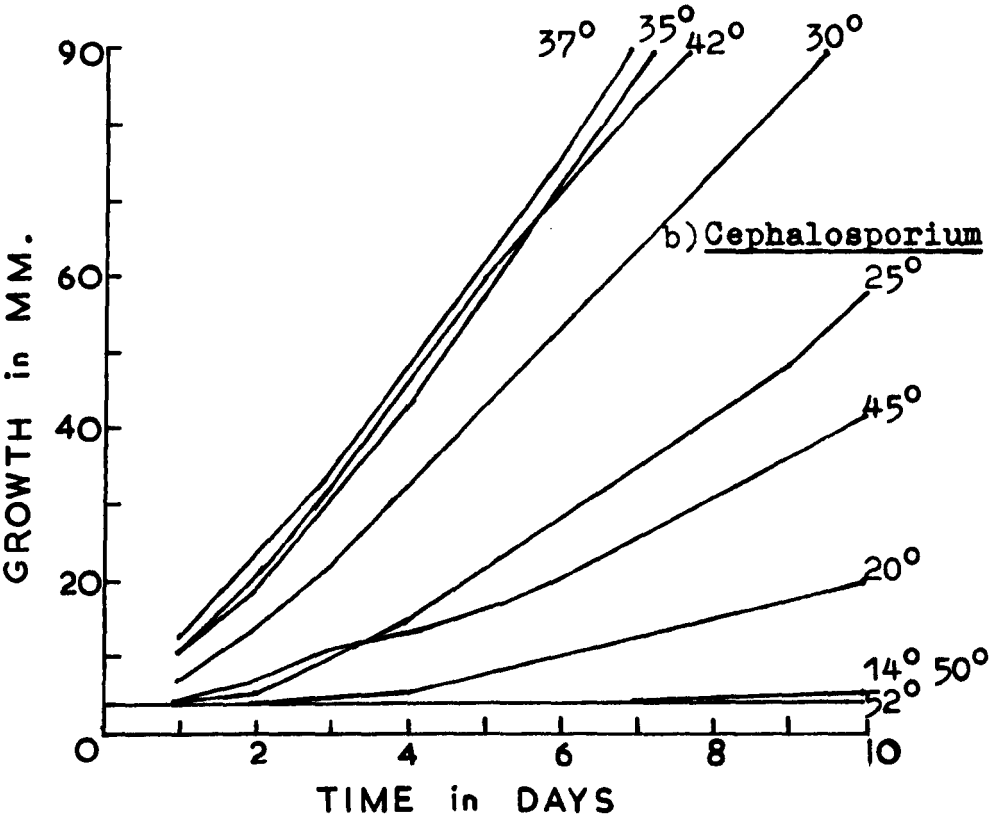
Cardinal temperatures °C.	min.	opt.	max.
	14	37	48

The temperature-growth relationships are shown in Figure 18a A trace of growth was recorded at 14°C and only slight growth occurred at 20°C. At 25°C the growth rate was still relatively slow but above 30°C it rapidly escalated. Between 35°C and 42°C there was a rapid rate of growth with the optimum occurring at 37°C. At 45°C and 48°C growth was greatly reduced and at 50°C no growth or spore germination was detected.



a) Cephalosporium sp. 1

Figure 18 **Temperature-growth relationships**



b) Cephalosporium sp. 2

Cephalosporium sp. 2

Cardinal temperatures °C.	min.	opt.	max.
	14	37	50

The temperature-growth relationships of this species are very similar to those reported above (see Figure 18b) for Cephalosporium sp. 1. However, the former species grew much faster at the lower temperatures (20°, 25° and 30°C) when compared with the latter species. A similar group of growth curves was obtained between the temperature limits of 35°C and 42°C, and this could be delimited as the optimum temperature range for growth, with 37°C being the optimum temperature. The growth rate fell rapidly at 45°C and 48°C, and at 50°C only spore germination was present.

These two species were greatly affected by temperatures above 42°C and hence they can be classed as weak thermotolerants, especially as they are both able to grow at relatively low temperatures.

Chrysosporium sp. 1

Cardinal temperatures °C.	min.	opt.	max.
	20	42	55

The temperature-growth relationships of this species are presented in Figure 19a). Growth at 20°C was very slow but above 25°C the growth rate rapidly increased. Between 35°C and 45°C an extremely rapid growth rate was maintained with the optimum occurring at 42°C. At 50°C growth was still relatively fast and similar to that at 30°C, but above the former temperature growth was markedly slowed down and at 55°C only spore germination occurred.

Chrysosporium sp. 2

Cardinal temperatures °C.	min.	opt.	max.
	25	40-45	55

No growth occurred at 20°C or 22°C but after an initial lag period slow growth was recorded at 25°C (see Figure 19b) Above this temperature the growth rate rapidly increased and an optimum was reached over a temperature range of 40°C to 45°C. Growth was still rapid at 50°C but above this temperature the growth rate dramatically decreased and only slight growth was present at 52°C. At 55°C only spore germination was detected.

Chrysosporium sp. 1 and 2 can both be classed as true thermophiles but the latter species would appear to be more strongly thermophilic than Chrysosporium sp. 1,

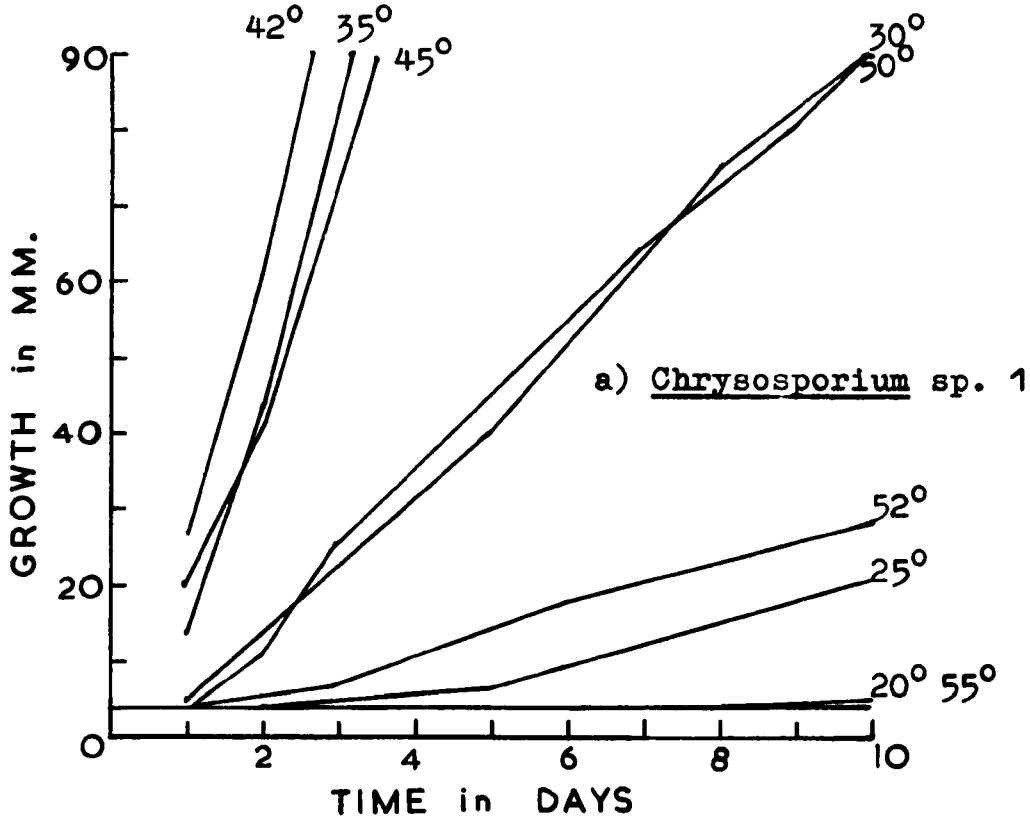
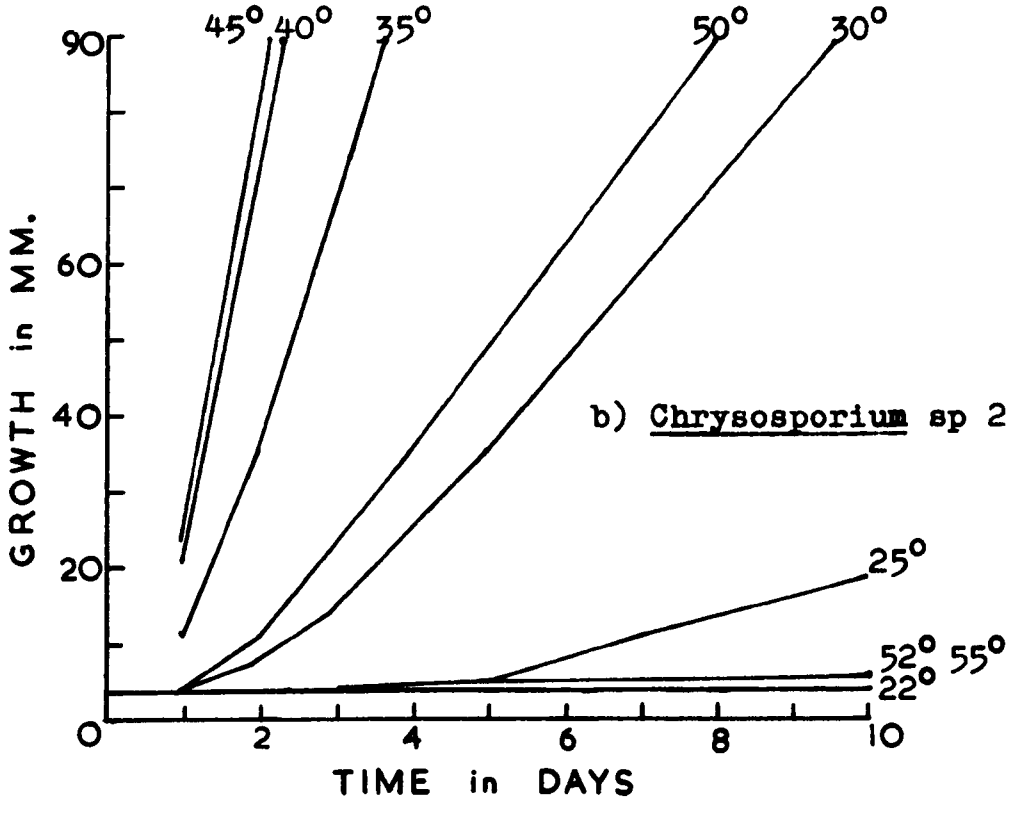


Figure 19 **Temperature-growth relationships**



due to its high minimum temperature for growth and also its slightly higher optimum temperature range. Nevertheless, above 50°C the growth rate of Chrysosporium sp. 2 was more markedly affected than that of Chrysosporium sp. 1. Hence, certain anomalies do exist at the higher temperature levels when growth rates are examined.

The fact that certain species of the genus Chrysosporium are capable of growth over a wide temperature range has been reported by Carmichael (1962) and Bergman and Nilsson (1966). The latter authors investigated the temperature-growth relationships of C. lignorum and reported a temperature range of 10°C to 50°C, with the optimum occurring at 40°C. During the present study an isolate of C. pruinosum was examined and was found to grow between 10°C and 45°C. However, all the species so far reported are weak thermotolerants and grow very slowly above 40°C and relatively rapidly below 20°C. The two species investigated during the present study can, therefore be separated from the remainder of the genus by their temperature-growth relationships, i.e. true thermophiles.

Geotrichum sp.

Cardinal temperatures °C.	min.	opt.	max.
	10	35-42	48

The temperature-growth relationships of Geotrichum sp. are presented in Figure 20a). A trace of growth was recorded at 10°C and growth was still limited at 12°C and 14°C. However, above 16°C the growth rate markedly increased and extremely rapid growth occurred over a wide temperature range, i.e. 18-45°C, with the optimum growth occurring at 35-42°C (maximum growth at these temperatures was completed after twenty-four hours incubation). At 48°C growth suddenly ceased and only spore germination occurred. This sudden and dramatic decrease in growth at temperatures above 45°C is probably due to heat-labile enzymes which operate successfully at elevated temperatures, but above a certain temperature enzyme inhibition (breakdown) is complete and development is halted.

This would appear to be an extremely versatile species growing successfully over a wide temperature range but the thermophilic properties of this species are limiting, despite the relatively high temperatures for optimal growth. Geotrichum candidum has been isolated from paper-mill slimes by Everleigh and Brewer (1963) where warm conditions prevailed. It is thought that this species is weakly thermotolerant but unable to grow above 40°C. Cooney and Emerson (1964, p.108) also mentioned the

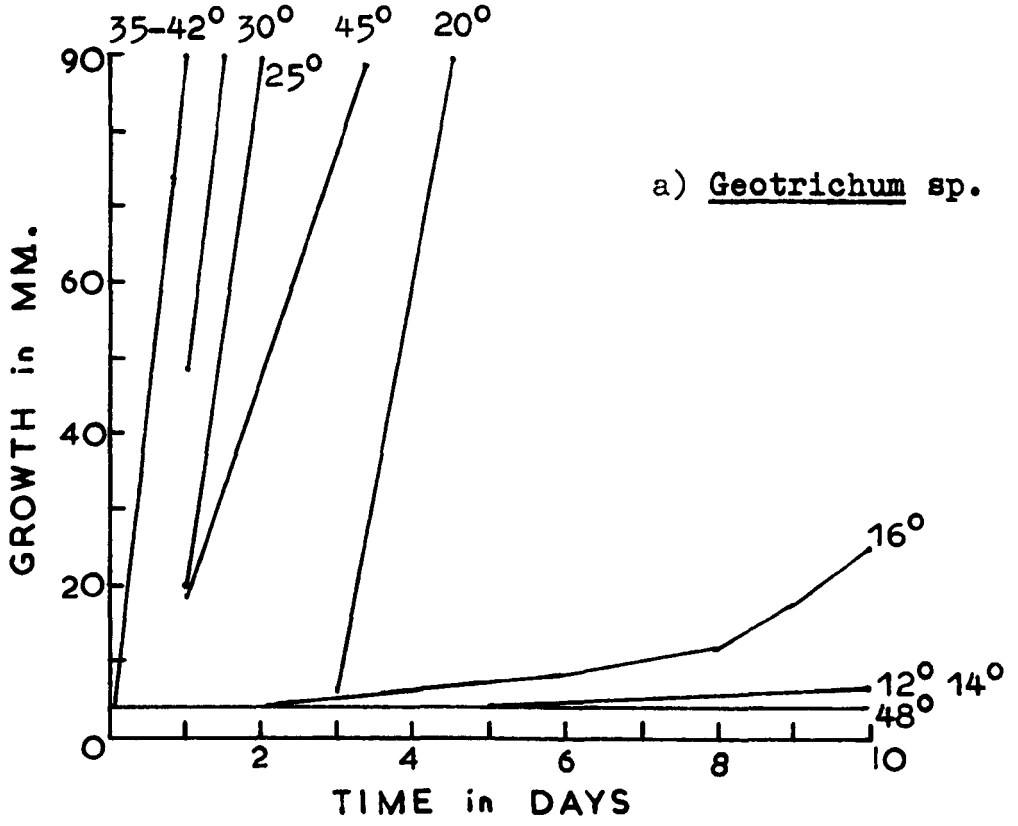
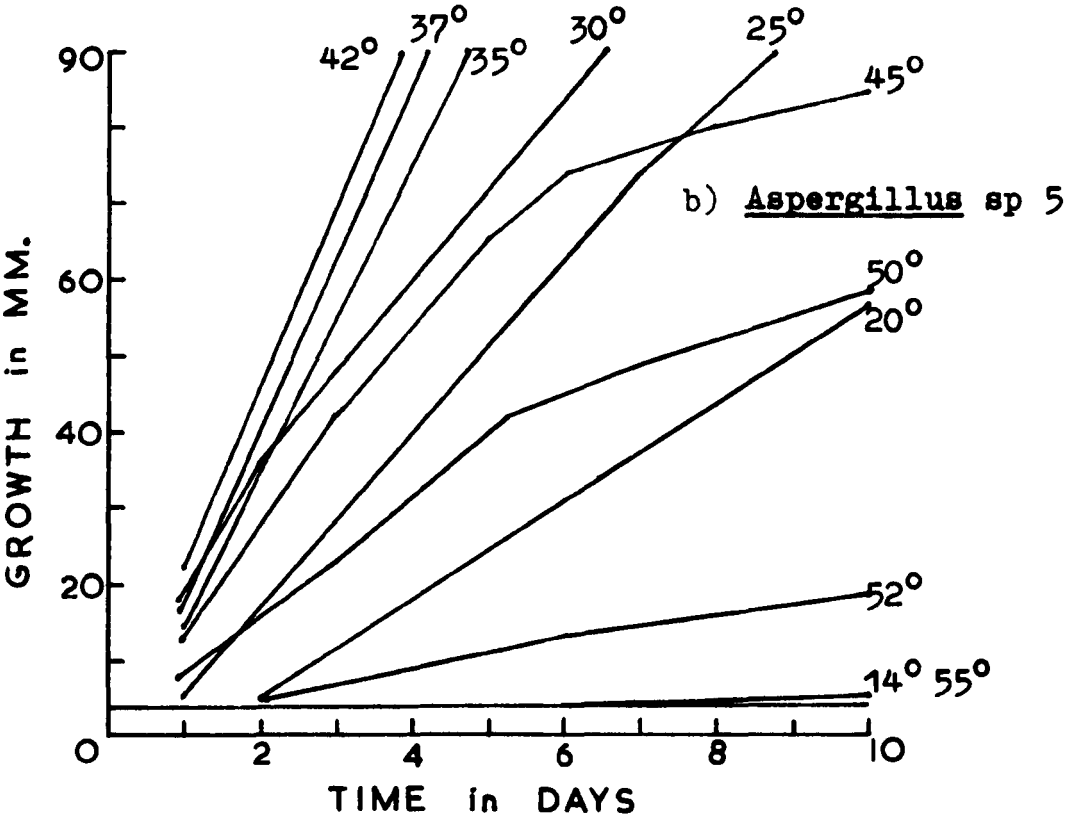


Figure 20 **Temperature-growth relationships**



occurrence of this species in self-heating hay.

Humicola insolens

Cardinal temperatures °C.	min.	opt.	max.
	20	42-45	52

Three isolates of this species were studied and all were found to have similar temperature-growth relationships. At 20°C growth was very slight and below this temperature growth was not recorded. At 30°C growth was substantial and between 35°C and 50°C a rapid growth rate was maintained, with optimum growth occurring at 42-45°C. Growth was reduced markedly at 52°C and absent at 55°C.

Cooney and Emerson (1964) recorded 23°C as the minimum temperature for growth of this species and 55°C as the maximum, with the optimum occurring between 35°C and 40°C. However, Craveri et al. (1967) reported growth of H. insolens at 20°C and also slight growth at 55°C. Hence there would appear to be significant variations in the temperature-growth relationships of different strains of H. insolens. Nevertheless, because of the ability of certain strains of this species to grow at 20°C and not at 55°C it can be considered to be weakly thermophilic.

Humicola sp. 1

Cardinal temperatures °C.	min.	opt.	max.
	16	45	52

A trace of growth was recorded at 16°C and at 20°C growth was still very slight. Above this temperature the growth rate increased relatively rapidly and a clear optimum occurred at 45°C. Above 48°C growth rapidly declined and at 52°C only spore germination was detected. At temperatures below 45°C, long lag periods occurred and only after four or five days was the optimum growth rate reached (see Figure 21a). This species must be considered to be a thermotolerant because of its ability to grow below 20°C, although growth at these temperatures was at a very much reduced level.

Humicola sp. 2

Cardinal temperatures °C.	min.	opt.	max.
	18	40	52

The temperature-growth relationships of this species are presented in Figure 21b). At 18°C and 20°C growth was extremely slow, above 25°C growth rapidly increased and between 30°C and 45°C a rapid growth was maintained. Optimum growth was reached at 40°C and above 45°C the growth rapidly declined. At 52°C only spore germination

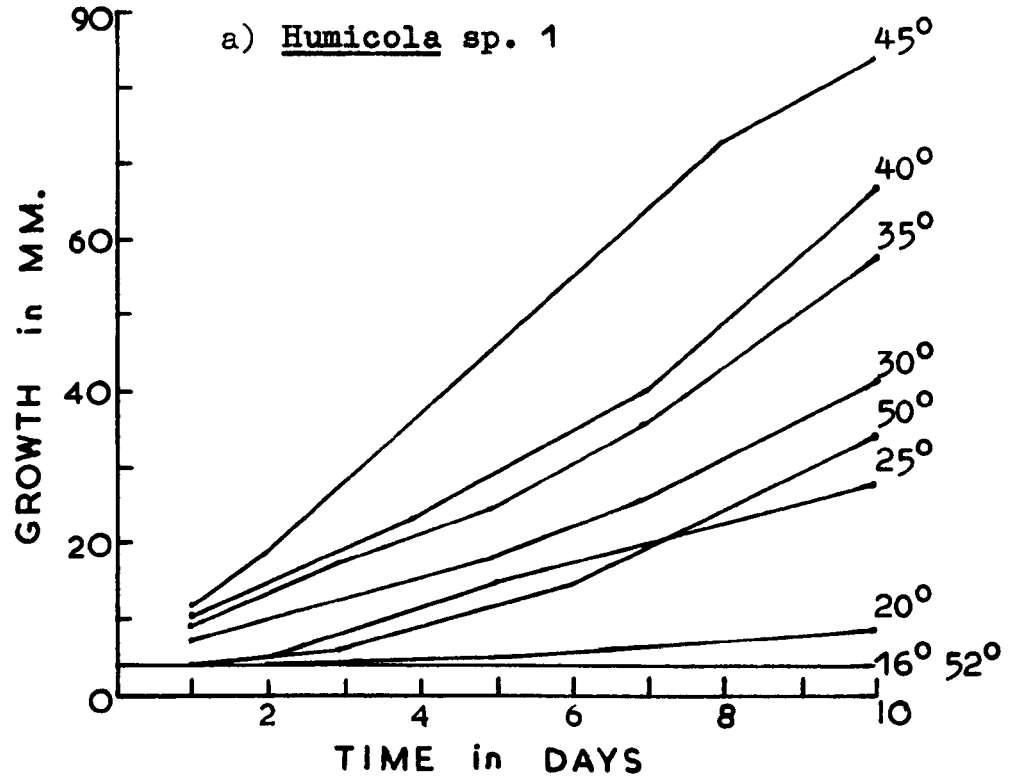
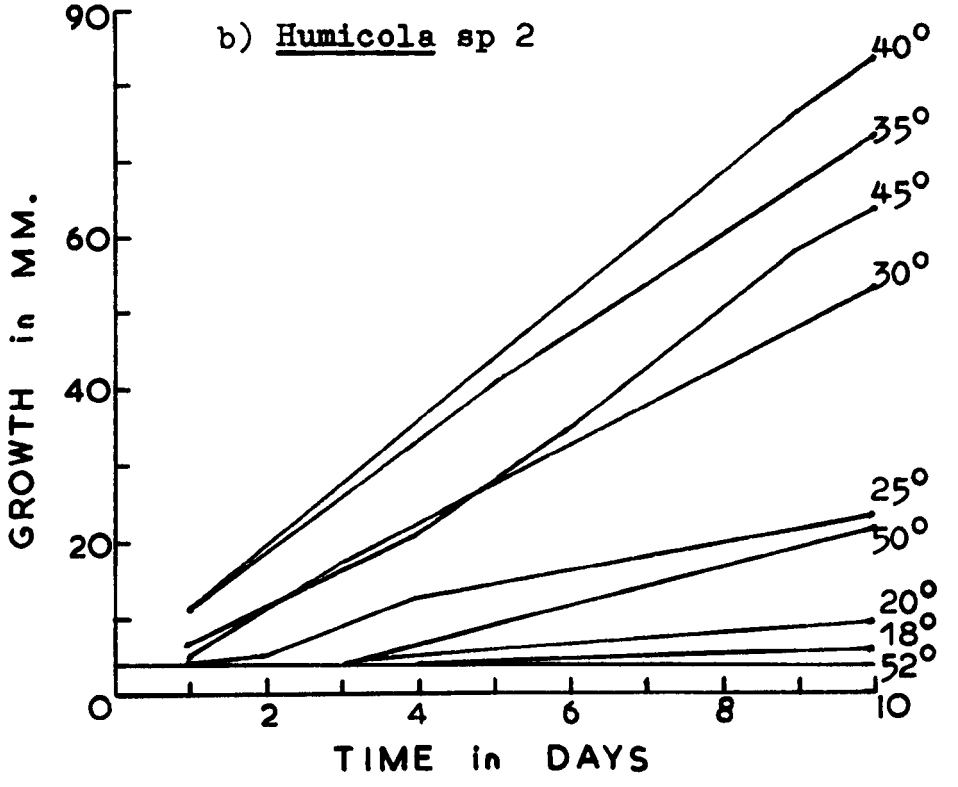


Figure 21 **Temperature-growth relationships**



was recorded.

The inability of this species to grow below 18°C and the very slight amount of growth recorded at 18-20°C would seem to place this species on the borderline between a thermotolerant and a true thermophile.

Paecilomyces sp.

Cardinal temperatures °C.	min.	opt.	max.
	12	35-37	50

At 12°C slight growth was recorded and above this temperature the growth rate steadily increased, with rapid growth between 30°C and 40°C. Optimum growth occurred at 35-37°C, and above 42°C the growth rate rapidly declined and growth was absent at 52°C. This species has a wide temperature range for growth similar to that of a number of thermotolerants.

Küster and Locci (1964) isolated a Paecilomyces sp. from peat which had similar cardinal temperatures for growth.

Penicillium piceum

Cardinal temperatures °C.	min.	opt.	max.
	12	37-40	48

P. piceum was found to grow over a wide temperature range, although the maximum temperature for growth (i.e. 48°C) was relatively low for the majority of thermotolerant species. This species grew slowly at all temperatures with optimum growth occurring at 37-40°C. Above 42°C there was a dramatic decline in the amount of growth produced. This species has been isolated from stored food products (hay, seeds, etc.) and from animal infections and its thermotolerant properties may enable P. piceum to exploit these habitats.

Penicillium sp. 1

Cardinal temperatures °C.	min.	opt.	max.
	14	37	52

The temperature-growth relationships of this species are presented in Figure 22a). At 14-18°C growth was extremely slow, but above 20°C the growth rate rapidly increased and between 30°C and 42°C the growth rate was extremely fast. Optimum growth occurred at about 37°C, although at 40°C and 42°C the growth rate was initially much faster than at the former temperature and only "tailed-off" during the later stages of incubation.

Penicillium sp. 1 can be classified as a thermotolerant

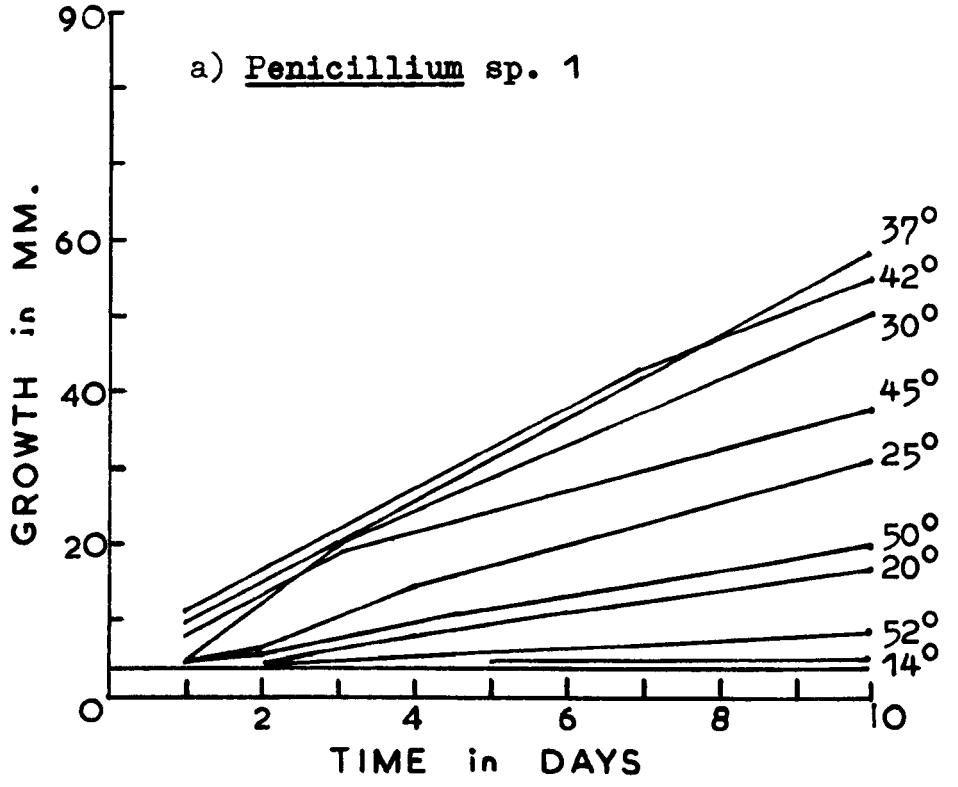
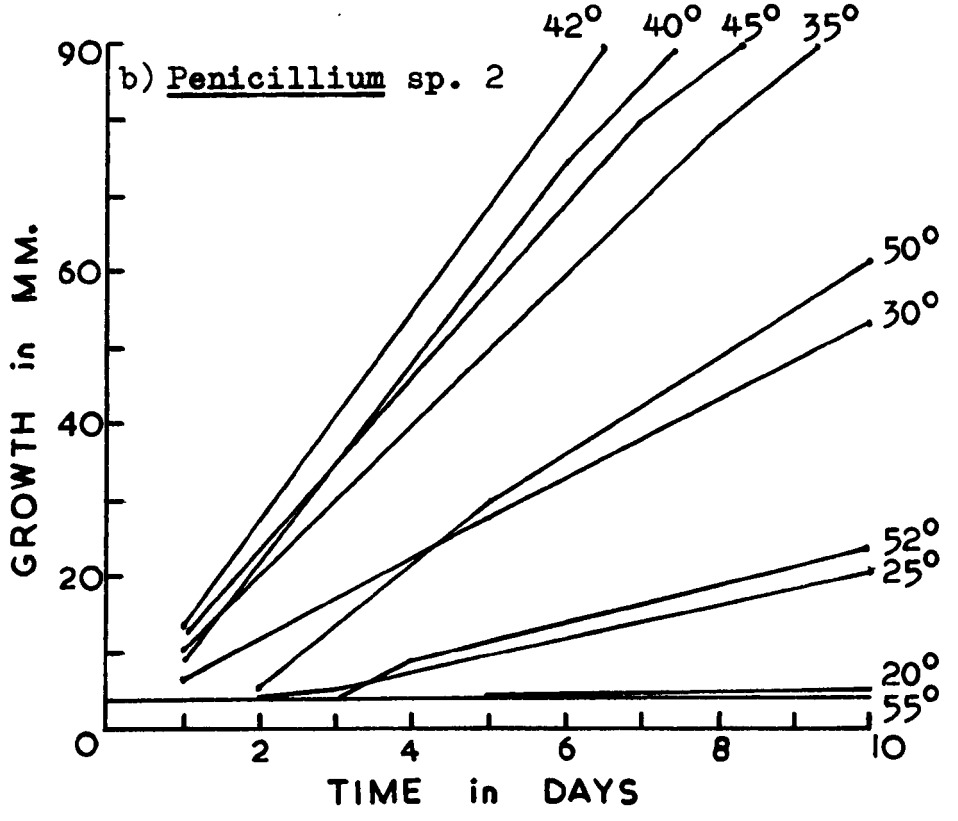


Figure 22 **Temperature-growth relationships**



species, having a wide temperature range for growth. Strong thermophilic tendencies are present, however, in view of the significant amount of growth recorded at 50°C and to a lesser extent at 52°C.

Penicillium sp. 2

Cardinal temperatures °C.	min.	opt.	max.
	20	42	55

The temperature-growth relationships of this species are presented in Figure 22b). A trace of growth was recorded at 20°C but was absent at lower temperatures. Above 25°C there was a rapid increase in the growth rate and an optimum temperature range occurred between 35°C and 45°C. A clear optimum was discernable at 42°C. At 50°C a relatively long lag period occurred but after several days an optimum growth rate was reached and growth at this temperature was in excess of that obtained at 30°C. At 52°C and 25°C the growth rates were similar and relatively slow, and at 55°C only spore germination was apparent.

Because of the inability of Penicillium sp. 2 to grow below 20°C and its ability to grow successfully at high temperatures, this species can be regarded as being a true thermophile.

Scolecobasidium sp.

Cardinal temperatures °C.	min.	opt.	max.
Strain 1	16	40	52
Strain 2	14	42	52
Strain 3	14	37-42	52

Three strains of this species, from different coal spoil tips were examined. The temperature-growth relationships of strain 1 are presented in Figure 24a). Strain 1 failed to grow at 14°C but the others grew extremely slowly at this temperature. Below 20°C the growth rate of all the strains were consistently slow. Above 20°C the growth rate increased rapidly and over a wide temperature range (i.e. 30-45°C) a rapid growth rate was maintained. Above 48°C the growth rate was markedly reduced and at 52°C only spore germination occurred.

There were slight differences in the optimum temperatures for growth of the three strains but in general the temperature-growth relationships of these strains were similar. Strain 3 was the fastest growing strain at the lower temperatures and was generally more active over the whole temperature range. This may be a reflection of the temperature gradients which exist between the warm areas

of different coal spoil tips, each strain adapting to the temperature conditions in its immediate environment.

Scolecobasidium sp. is a thermotolerant species which grows only slowly at temperatures below 25°C, but at elevated temperatures (30-45°C) grows extremely rapidly. It can therefore be separated from the weak thermotolerant species on the basis of these conditions.

Scopulariopsis sp.

Cardinal temperatures °C.	min.	opt.	max.
	14	40	50

At 14°C and 16°C slight growth was recorded but above these temperatures the growth rate steadily increased. Between 30°C and 42°C growth was rapid with the optimum occurring at 40°C (see Figure 23a). Above 42°C the growth rate decreased considerably. Growth was still apparent at 50°C but absent at 52°C.

Hyphomycete sp. (Calcarisporium sp.)

Cardinal temperatures °C.	min.	opt.	max.
	16	40	50

The temperature-growth relationships of this species are presented in Figure 23b). A trace of growth occurred at

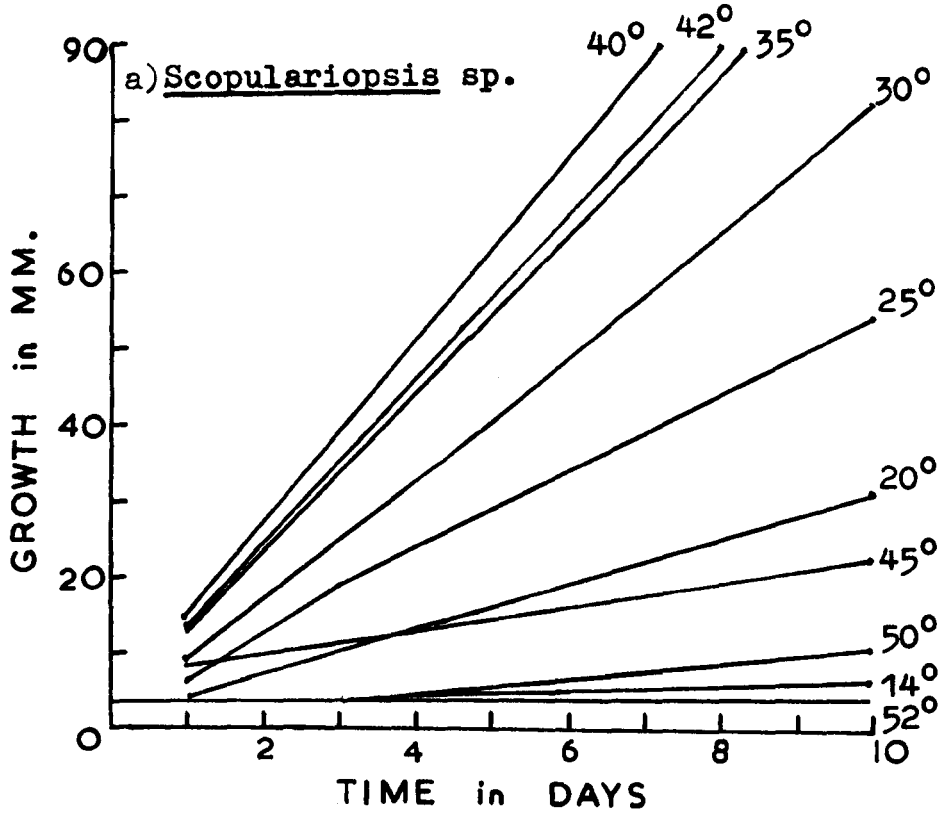
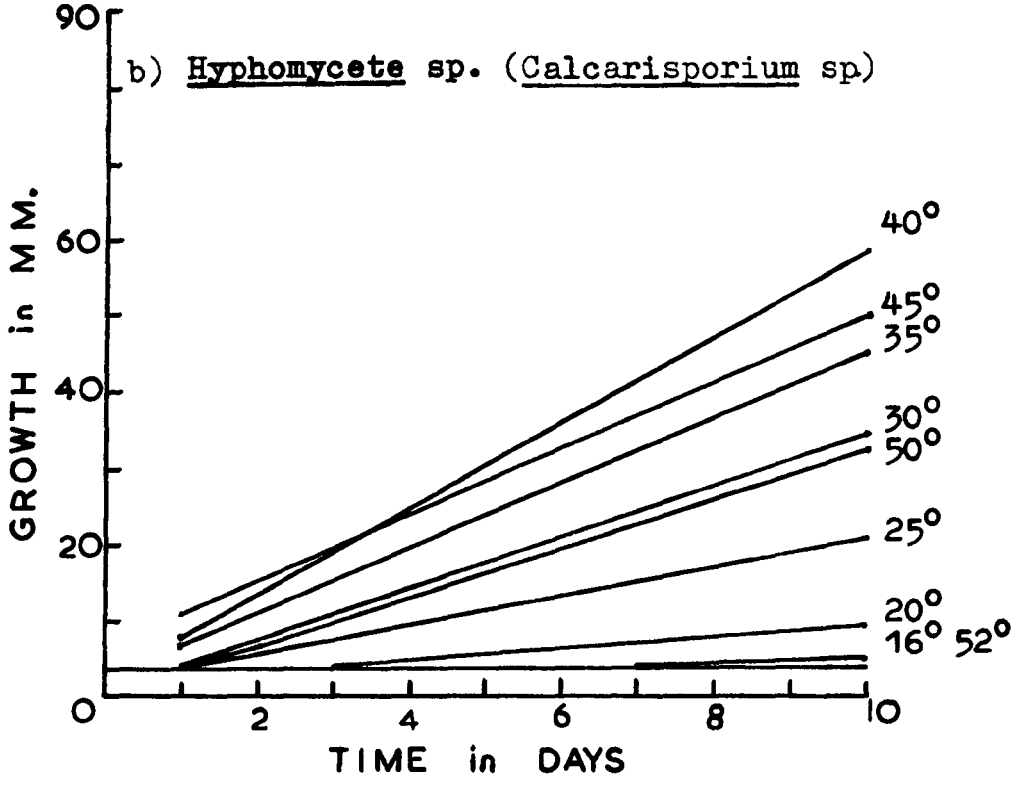


Figure 23 **Temperature-growth relationships**



16°C and 18°C, and at 20°C growth was still slight. Above this temperature there was a steady increase in the growth rate and an optimum growth range occurred at 35-45°C, with a well defined optimum at 40°C. Growth was still relatively rapid at 50°C, compared with that at 30°C. However, above 50°C growth suddenly ceased and no growth was recorded at 52°C.

These two species can both be classified as thermotolerants although the latter species has stronger thermophilic properties than Scopulariopsis sp. This was evident by the effect of high temperatures (i.e. 45-50°C) on the growth rates of these two species. The Hyphomycete sp. grew well at these temperatures in comparison with the other species (see Figure 23b). However, both failed to grow above 50°C, probably due to the destruction of heat-labile enzymes over a small temperature change (especially the Hyphomycete sp. at 50-52°C).

Sporotrichum thermophile

Cardinal temperatures °C.	min.	opt.	max.
	18	42-45	55

Three strains of S. thermophile were examined and all had similar temperature-growth relationships.

A small amount of growth was recorded at 18°C, and above 22°C the growth rate rapidly increased. Optimum growth occurred between 42°C and 45°C, although a rapid growth rate was maintained between 30°C and 50°C. No growth was observed above 55°C but at this temperature the amount of growth was still significant.

These results are in good agreement with those reported by Bergman and Nilsson (1966, 1967), who determined the optimum temperature at 45°C and the maximum at 55°C. However, Henssen (1957) observed no growth on certain media at 47-48°C, although very slight growth occurred on potato dextrose agar. There would appear to be therefore, considerable differences in the temperature-growth relationships of various strains of S. thermophile. However, the ability of this species to grow below 20°C would exclude it from being a true thermophile, and indeed, Cooney and Emerson (1964) concluded that "the species is very near the limit of our definition of a true thermophile".

Thermoidium sulphureum

Cardinal temperatures °C.	min.	opt.	max.
	25	45	55

Three strains of this species were examined and were found to have similar cardinal temperatures for growth, although one strain in particular had a significantly high growth rate than the other two. Growth was extremely slow at 25°C and at 35°C the amount of growth produced was still relatively small. However, between 40°C and 50°C a rapid growth rate was maintained with the optimum occurring at 45°C. Above 50°C there was a rapid decline in the growth rate and no growth was detected at 57°C.

Similar temperature characteristics were reported by Cooney and Emerson (1964, p.102), although Crisan (1959) and Craveri et al. (1967) recorded a narrower temperature range for this species. However, T. sulphureum can be regarded as a true thermophile due to the high minimum and optimum temperatures for growth. However, this species is more sensitive to temperatures above 50°C than a number of other true thermophiles and, therefore, probably not as strongly thermophilic.

Thermomyces lanuginosus

Cardinal temperatures °C.	min.	opt.	max.
	28	45-50	60

Very slight growth was recorded at 28°C and the growth

rate was still slow up to 40°C. Between 42°C and 55°C the growth rate was extremely rapid and optimum growth occurred at 45-50°C. Even at 57°C and 60°C the amount of growth produced was still appreciable. However, at 62°C growth was absent.

Cooney and Emerson (1964, p.87) recorded similar temperature-growth relationships, although growth below 30°C has not previously been reported. The growth of this species at 60°C, which has also been noted by Craveri et al. (1967) and Maheshwari (1968), makes it one of the strongest thermophiles so far studied.

Torula thermophila

Cardinal temperatures °C.	min.	opt.	max.
	20	42	55

A trace of growth occurred at 20°C but the growth rate progressively increased above this temperature, and between 30°C and 50°C it was extremely rapid. Optimum growth was recorded at 42°C. Above 50°C there was a rapid decline in the growth rate and growth was barely detectable at 55°C, and completely absent at 57°C.

The isolates examined by Cooney and Emerson (1964, p.92) had a minimum temperature for growth at 23°C, an

optimum at about 40°C and a maximum at 58°C. Hence, there would appear to be slight differences between strains of T. thermophila concerning their cardinal temperatures for growth. This species can be regarded as a true thermophile but because of its low minimum temperature for growth, it would appear to be weakly thermophilic.

Tritirachium sp.

Cardinal temperatures °C.	min.	opt.	max.
	16	37-42	55

The temperature-growth relationships of Tritirachium sp. are presented in Figure 24b). At 16°C only a trace of growth was detected and up to 20°C (and even 25°C) the growth rate was very slow. Above 25°C growth rapidly increased with the growth rate being extremely rapid between 35°C and 45°C. An optimum growth range occurred at 37-42°C. At 50°C growth was still significant but at 55°C only slight growth occurred. Growth was not present at 57°C.

This species has a very wide temperature range and has the ability to grow well at the higher temperatures. It would, therefore, appear to have strong thermophilic tendencies despite its ability to grow below 20°C.

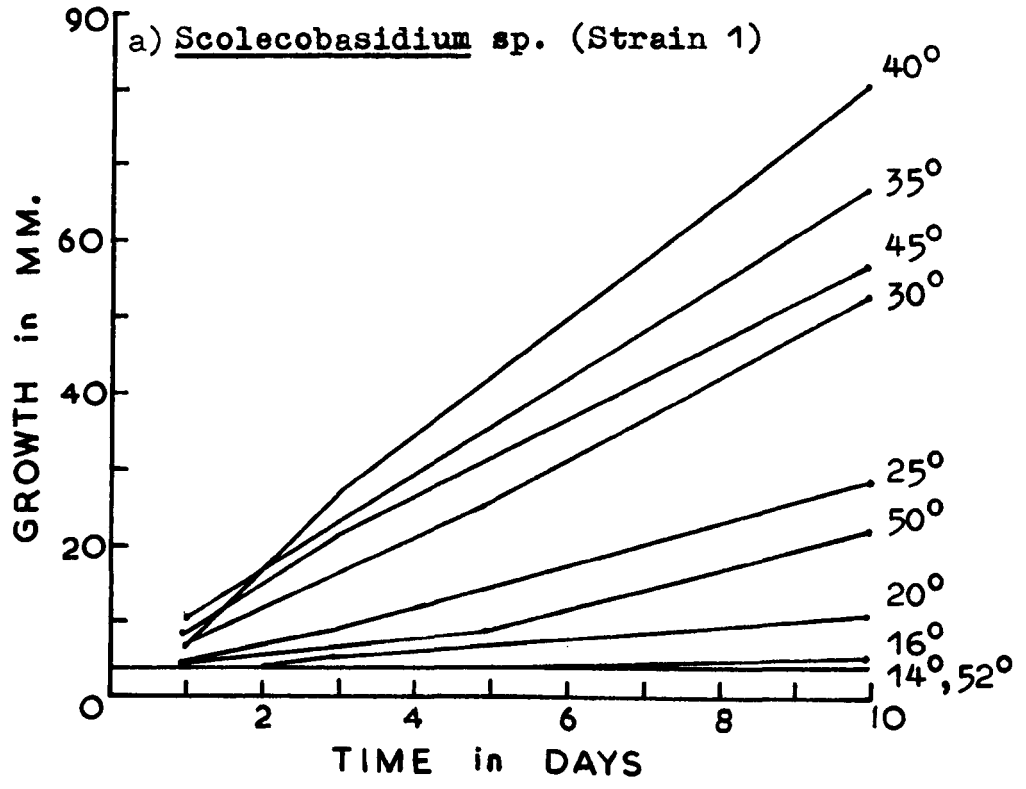
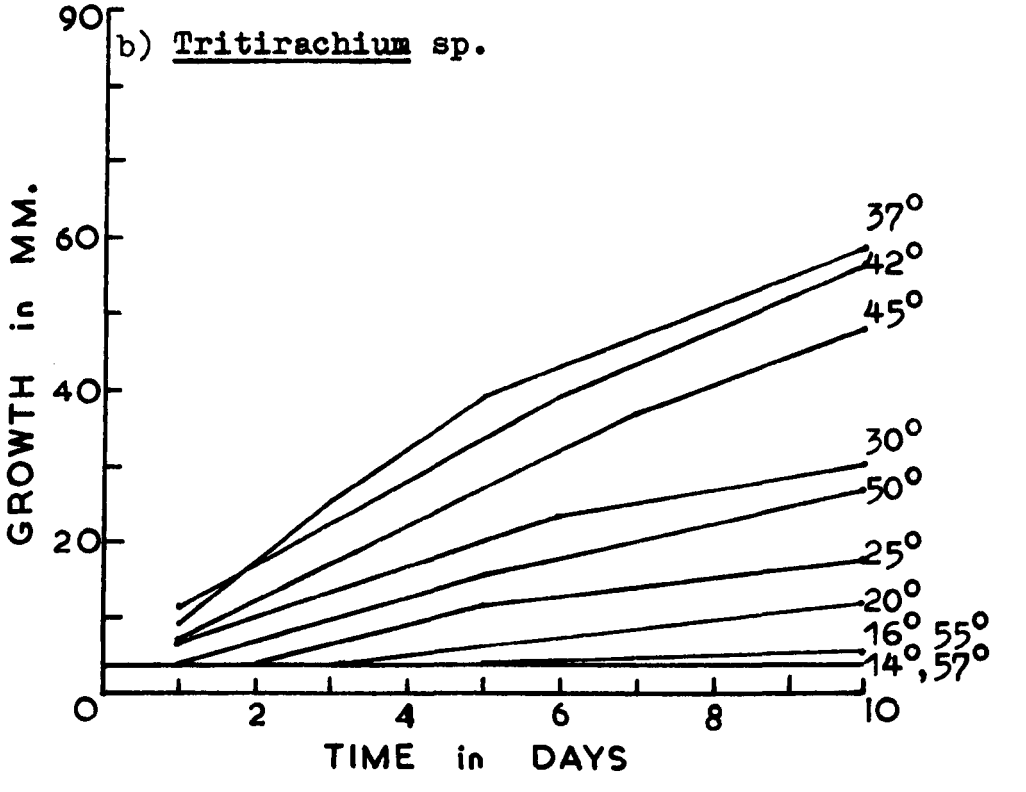


Figure 24 **Temperature-growth relationships**



Discussion

From an analysis of the temperature-growth relationships of the species investigated it is possible to propose several broad groupings, viz:

1. Strong thermophiles

Those species which have high minimum temperatures for growth, i.e. no growth below 25°C, and which correspondingly have high maximum temperatures.

2. Weak thermophiles

Those species which have relatively low minimum temperatures for growth, i.e. at or just above 20°C, and which, therefore, qualify as true thermophiles according to the definition proposed by Cooney and Emerson. These species usually grow well at or above 50°C but rarely above 55°C.

3. Strong thermotolerants

Those species which grow sparingly at and slightly below 20°C, and which have their minimum temperature for growth near to this temperature but which cannot be classified as true thermophiles. These species usually grow reasonably well at 50°C and above.

4. Other thermotolerants

Those species that can grow well over a wide

temperature range and which have their optima at or around 40°C but which are also able to grow at temperatures well below 20°C. These species usually grow very slowly (or not at all) at 50°C and above.

Group 1 includes: Chaetomium thermophile, Chrysosporium sp. 2, Myriococcum albomyces, Rhizopus sp. 1, Talaromyces duponti, T. emersonii, Thermoascus aurantiacus, Thermoidium sulphureum and Thermomyces lanuginosus.

The minimum temperatures for growth of these species varies from 25-30°C, the optimum lies at about 45°C (and never below) and the maximum at 55°C or above. In fact, a large proportion (two thirds) of these species are able to grow at 60°C.

Group 2 includes: Allescheria terrestris, Chrysosporium sp. 1, Dactylomyces crustaceus, Humicola insolens, Mucor miehei, M. pusillus, Penicillium sp. 2, Talaromyces sp. and Torula thermophila.

The majority of these species are able to grow at 20°C, although not below it. Their optimum for growth varies from 37-45°C and for a large proportion of the species lies at or about 42°C. Growth of these species

was rarely recorded above 55°C and the amount of growth occurring at this temperature was usually restricted. It is interesting to note that the two species with the highest minimum temperatures for growth (i.e. A. terrestris and M. miehei at 22°C), also have the highest optimum and maximum temperatures.

Group 3 includes: Chaetomium sp. 2, Dactylomyces thermophilus, Humicola spp. 1 and 2, Sporotrichum thermophile, Talaromyces sp. and Tritirachium sp.

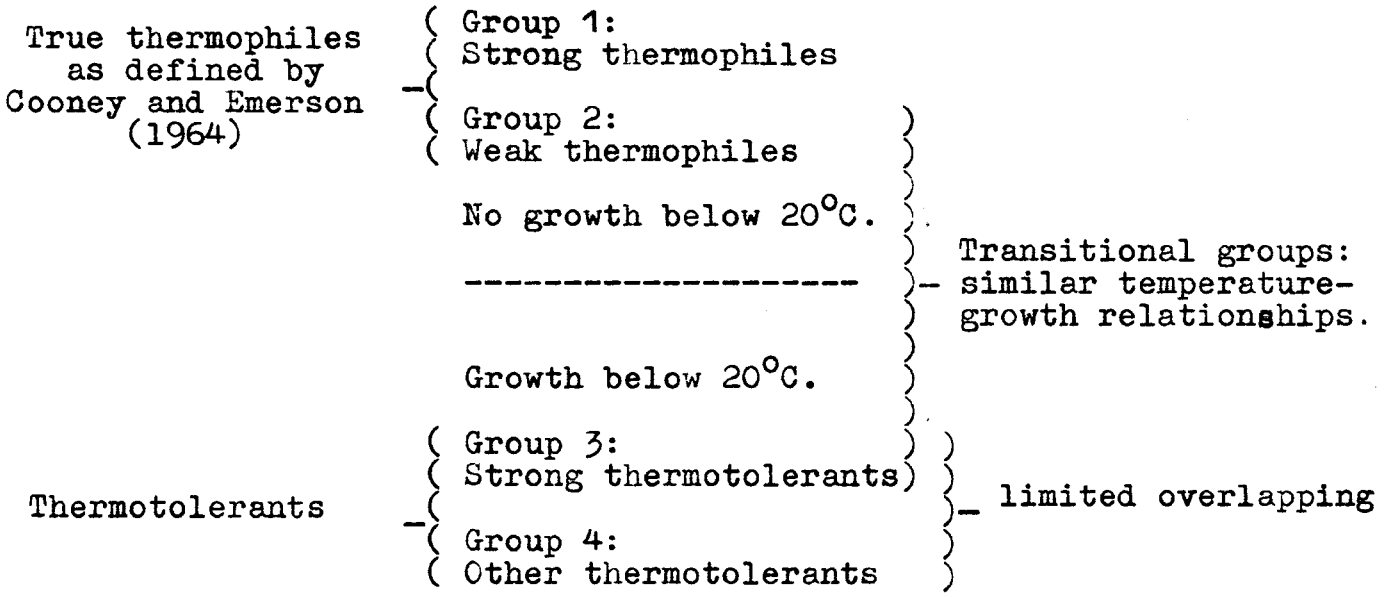
All these species grow very slowly at 18°C and 20°C and usually do not grow below 18°C. They are all able to grow at or above 50°C and a number have their maximum temperature for growth at or about 55°C. The optimum temperature for growth of the species varies from 37-45°C and in the majority of cases lies close to 42°C. A comparison of the temperature-growth relationships of the fungi of groups 2 and 3 shows very close similarities, especially in their optimum and maximum temperatures for growth. In fact, the main point of discrepancy lies in their minimum temperatures for growth. Between these two groups the definition of thermophily intrudes and separates them into two artificial groupings, and it may be that strains of some of the species are transitional

between these two groups. Also, these groups may be natural steps in the evolution of thermophily, and the temperature of a habitat, if continually high, may change thermotolerant fungal strains and eventually render them unable to grow at normal temperatures, i.e. below 20°C.

Group 4 includes: Absidia corymbifera, A. ramosa, Aspergillus fumigatus, A. nidulans, A. terreus, Aspergillus spp. 1 to 5, Cephalosporium spp. 1 and 2, Chaetomium sp. 1, Geotrichum sp., Mortierella sp., Paecilomyces sp., Penicillium piceum, Penicillium sp. 1, Rhizopus spp. 2 and 3, Scolecobasidium sp., and Hyphomycete sp. (Calcarisporium sp.).

These species grow well over a very wide temperature range, i.e. they grow over the normal mesophilic range of 10-35°C, but are also able to grow up to 45°C and sometimes 55°C. These high maximum temperatures for growth may be of tremendous importance in determining the habitats in which thermotolerant species occur, e.g. in stored food, waste materials, warm-blooded animals and in industrial areas. The pressures of these environments may select out those species most suited, i.e. species able to grow well at low and high temperatures, which will be especially important in habitats

Classification of Thermophilous Fungi



where wide fluctuations occur. Certain species of this group do, however, grow only slowly at temperatures below 20°C, e.g. Scolecobasidium sp., Hyphomycete sp., and show to some extent similarities with the group 3 fungi, although in general the former species are more susceptible to temperatures above 50°C.

The groups can be seen therefore, to form parts of a gradient where minimum growth temperature is the deciding factor for position on this gradient. The groups do, in fact, grade or merge into each other and at points overlap, and hence the definition of thermophily becomes more obscure (or arbitary). However, it is thought that the group 1 fungi constitute the true thermophiles and these fit the temperature requirements for thermophilic fungi as suggested by Küster and Locci (1964) and Craveri et al. (1964, 1967). Groups 2 and 3 fungi constitute a transitional stage between true thermophily and general thermotolerance, the latter term embracing those species included in group 4.

An important consideration in determining temperature-growth relationships is the period of time over which incubation is carried out. For example, at low and high

temperatures a number of species were found to have long lag phases before growth occurred, and therefore, short incubation periods may limit or give false impressions of the cardinal temperatures of these species. As mentioned earlier (see general discussion, Chapter II), the cardinal temperatures of a fungus determined under laboratory conditions may not reflect those which occur when the fungus grows under natural conditions. It may be that certain of the thermophilic fungi grow extremely slowly in the soil at temperatures below their minimum growth temperature, as measured in the laboratory using a relatively short incubation period. However, El-Abyad and Webster (1968a) found that the cardinal temperatures of several pyrophilous fungal species when grown on malt extract agar, were similar to those obtained when burnt soil was used as the growth medium.

Cochrane (1958) stated that subtropical strains of fungal species may have a higher temperature optimum than strains from temperate regions, and hence geographical isolates of the same species may differ in their temperature-growth relationships. This may be true of many of the species investigated in this study, where the temperature-growth relationships of a fungus reflect

the temperature conditions of their environment or habitat. In fact, several species were shown to have slight strain differences in their temperature requirements for growth, e.g. Talaromyces duponti, Thermoascus aurantiacus and Scolecobasidium sp.

The mechanisms of thermophily are as yet unknown and in the present study the reactions of fungal species to high temperatures varied considerably. For example, certain species grew well at a specific temperature but a small change of several degrees produced a dramatic and marked decline in the growth rate resulting in a negligible amount of growth being produced or a complete absence of growth, viz: Mortierella sp. - temperature change from 45-48°C, Trichophaea sp. (48-50°C), Chaetomium sp. 1 (50-52°C), Rhizopus sp. 1 (57-60°C), Thermoascus aurantiacus (60-62°C). This is probably due to a delicately balanced enzyme system, especially in those species with rapid growth rates which by virtue of this fact may produce more enzymes than are being broken down, until a critical stage is reached. This is the dynamic theory of thermophily as proposed by Allen (1950), enzymes being produced faster than they are destroyed at high temperatures. To explain this greater synthetic ability at

high temperatures, and poor or no growth at low temperatures, a higher coefficient of enzyme synthesis was proposed for thermophiles in comparison with mesophiles. The reactions of certain other species to high temperatures differed from this sudden metabolic breakdown, and growth gradually slowed down over a relatively large temperature range, and for these species there may be different mechanisms of thermophily operating, i.e. heat-stable enzymes. Brock (1967, 1969) has concluded that the cell membranes of thermophiles are most likely to be the crucial thermostable structures and he has reviewed the evidence in favour of this theory.

Cooney and Emerson (1964) stated that 60°C must be near the upper limit for the growth of fungi, and this would appear to be true in the light of the present results. This may be due to the organisation or composition of the enzyme systems of this taxonomic group, whereas other groups of organisms, e.g. bacteria, algae, members of which can grow at significantly higher temperatures than 60°C, may possess different metabolic systems or methods of adaptation to high temperatures.

CHAPTER V

TAXONOMIC STUDIES

OF

THERMOPHILOUS FUNGI

The complete list of fungi isolated during the present study will be reviewed and special reference will be given to unusual or interesting species on which taxonomic studies are rare or absent. These will be described in the same order as previously, i.e. Phycomycetes, Ascomycetes and Deuteromycetes. The line drawings of the organisms studied were made with the aid of a camera lucida and whenever feasible structures were drawn using oil immersion. Habitat views of the various stages were drawn directly from the agar surface using the low power of a phase-contrast microscope.

Phycomycetes

a) Mucoraceae

i. Absidia

Two species belonging to the genus Absidia were isolated from the soil and the air spora, and proved to be of relatively frequent occurrence.

A. corymbifera (Cohn) Saccardo

Synonym: A. lichtheimii (Lucet and Costantin) Lendner.
see Zycha, p.127, 1935.

The cultures examined were similar to those described by Lendner (1908, p.143) and Zycha, although two of the

strains examined produced irregular chlamydospores in and on the agar surface. They have not previously been observed for this species.

A. ramosa (Lindt) Lendner.

see Zycha, p.134, 1935.

The sporangiospores were found to vary in shape between the various isolates studied but the majority possessed mainly cylindrical spores. The rest of the morphology is similar to that described by Lendner (1908, p.144) and Zycha.

Both these species have been reported to cause diseases in guinea-pigs and cattle (Ainsworth and Austwick 1955). The latter authors isolated both species directly from the internal organs of diseased guinea-pigs and they recorded their frequent distribution in association with various animals. A. corymbifera and A. ramosa have also been isolated from hay and the air spora of farm buildings (Lacey and Lacey 1964) but their occurrence in soil has rarely been reported. They were isolated, sometimes frequently, from several different soils during the present investigation. Their true natural habitat, however, is obscure but Ainsworth and Austwick link this with their apparent involvement in

animal diseases. The thermotolerant nature of these two species may favour their development in the tissues of warm-blooded animals.

ii. Mucor

Mucor miehei Cooney and Emerson.

Mucor pusillus Lindt.

in Thermophilic fungi, pp.17-27, 1964.

M. pusillus is re-described by Cooney and Emerson and on the basis of several taxonomic points they propose a separate new species, M. miehei. The asexual stages of both species are essentially very similar but differences are proposed on the basis of their sexual morphology. M. pusillus is described as heterothallic and M. miehei as homothallic. Furthermore, the Zygospores of M. pusillus (45-63 μ in diameter) are generally larger than those of M. miehei (30-50 μ in diameter). Minor differences concerning colour and thickness of turf are also noted.

However, Sarbhoy (1968, p.27) after examining seventeen strains of M. pusillus and M. miehei concludes that the creation of the new species, M. miehei, is not justified on the basis of these 'strain' differences and

that it is necessary to regard this species as a synonym of M. pusillus. Indeed, Sarbhoy (1967) in a personal communication, after examining one of my strains of M. miehei (IMI 125,824) considered it to be a homothallic strain of M. pusillus. In his paper the author describes M. pusillus as being mostly pathogenic on animals but in the present study it was found to be an extremely ubiquitous species, probably forming part of the natural soil population. He also doubted the heterothallic nature of M. pusillus and in his key refers to it as a homothallic species.

A study of the present isolates of M. pusillus revealed great differences in their cultural characteristics. Colony colour was found to vary from grey to dark brown with turf of varying degrees of thickness and height. Several strains readily produced zygospores and their cultural characteristics approached those described for M. miehei. These strains were, however, very few in number compared to the vast amount of normal strains of M. pusillus obtained during sampling. In general, the homothallic strains of M. pusillus obtained could be readily distinguished from the standard strains by their much lighter colony colour, varying from white to light

grey, and the relative thinness of the turf produced. At low temperatures, 25-30°C, these strains produced colonies consisting of masses of zygospores with little or no production of sporangia. This is in complete contrast with normal strains of M. pusillus. Single-spore cultures of these strains continued to produce zygospores, evidence of their homothallic nature. Few of the strains of M. pusillus were able to produce zygospores, but the strains which had this capability could produce zygospores in single-spore culture. This would appear to be evidence of the homothallic nature of M. pusillus but because of its restriction to a small number of strains it can be concluded that the majority of strains are heterothallic.

It can be summarised, therefore, that homothallic strains distinguishable from M. pusillus (normal strains) by colony colour and texture, and by zygospore size were infrequently isolated and these strains approximate M. miehei Cooney and Emerson. The majority of strains of M. pusillus were found to be heterothallic but a few strains showed limited zygospore production in single-spore cultures, i.e. homothallic. It is undecided whether or not these morphological characteristics are

significantly different to warrant a separation into two distinct species. However, separation at least on a varietal standing is considered to be justified. The consistently stronger thermophilic properties of M. miehei (see Chapter IV) may also serve to delimit this species from M. pusillus. Slight differences were also observed in the suspensor morphology, those of M. miehei being shorter and more inflated than those of M. pusillus.

Mucor sp.

These strains were initially assigned to M. pusillus but a closer morphological examination revealed certain differences from the normal strains of M. pusillus. They were commonly isolated from a number of habitats.

Growth of this species is relatively slow, in comparison with strains of M. pusillus, at all temperatures but more specifically at the higher temperatures. Dense, dark grey to brown colonies are formed and morphologically the asexual stage is indistinguishable from that of M. pusillus. A closer microscopic examination of these strains reveals the presence of groups of chlamydospores or gemmae. In several instances clumps of oval spores were found to occur on the sporangiophore and occasionally these were associated with thin hyphal

extensions wound around the sporangiophores, seemingly to give the appearance of secondary mycelium (see Figure 25). However, other strains failed to show this secondary mycelium and large, spherical chlamydospores in groups were observed on the agar surface or in the aerial mycelium. All these stages stain heavily with cotton blue in lactophenol, easily being distinguished from the asexual structures. The presence of chlamydospores has never been reported in any of the previously studied strains of M. pusillus.

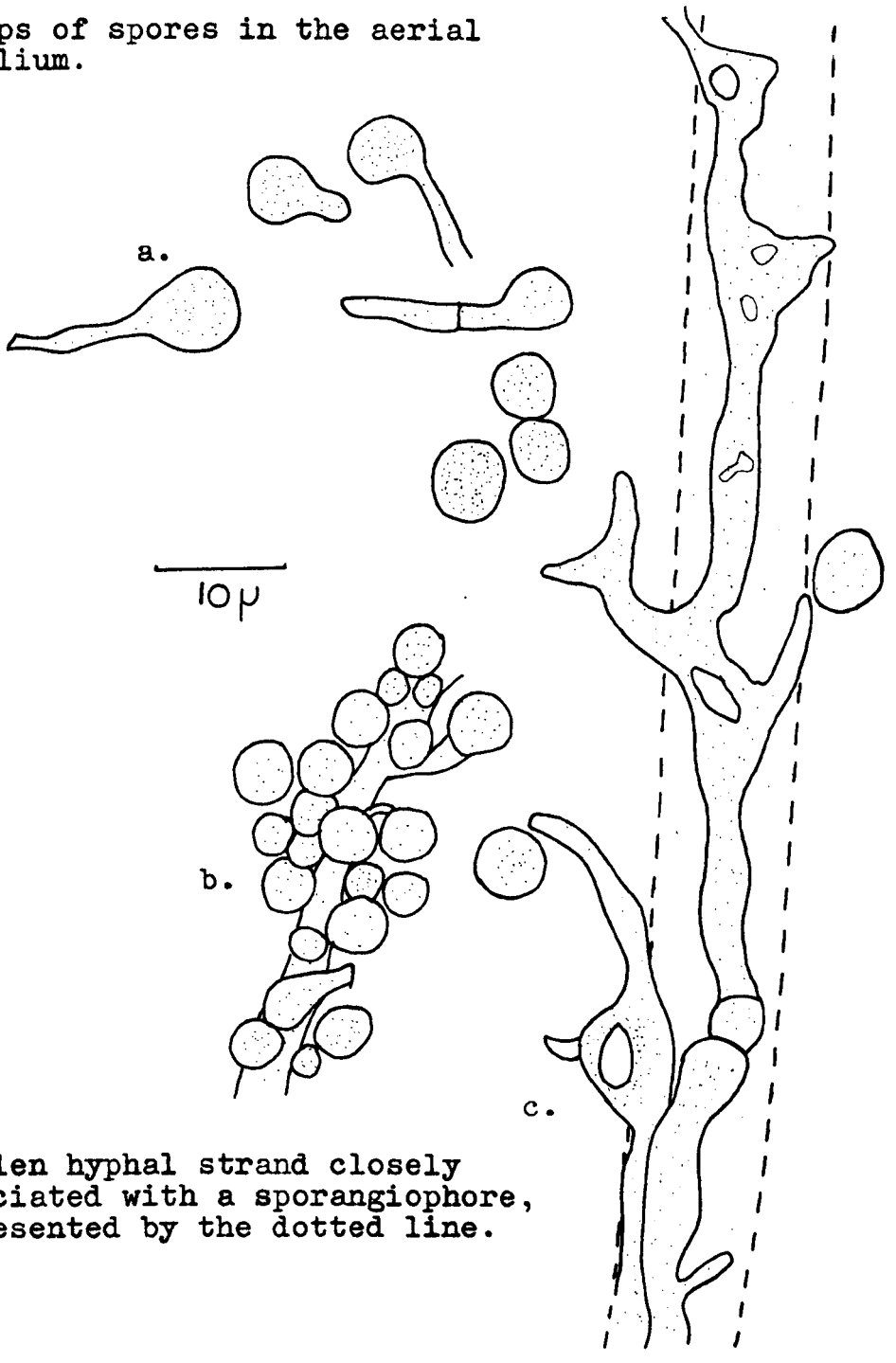
It may be supposed that these cells represent a mycoparasite associated with M. pusillus. However, careful separation of these cells and their subsequent sub-culturing produces similar Mucor cultures. The structures are not comparable with any of the described common mycoparasites of Phycomycetes but if they belong to a mycoparasite then this species must have a similar temperature-growth pattern to the Mucor species and would therefore be considered to have thermophilic properties. The cells are not specifically formed at any particular temperature but in general occur throughout the temperature range for growth of the Mucor. However, due to the lack of further information it is

Figure 25

Mucor sp.

a. Chlamydospores

b. Groups of spores in the aerial mycelium.



c. Swollen hyphal strand closely associated with a sporangiophore, represented by the dotted line.

thought that Mucor sp. represents strains of M. pusillus possessing globose or oval chlamydospores, 5-7 μ in diameter, occurring on the agar surface or in the aerial mycelium.

iii. Rhizopus

Three species belonging to the genus Rhizopus were isolated during the present study.

Rhizopus sp. 1

This species was isolated exclusively from coal spoil tips, particularly from the warm areas where it had a high frequency of occurrence.

On potato dextrose agar at 45°C Rhizopus sp. 1 grows extremely rapidly and completes radial expansion on a petri plate (9 cm. in diameter) in less than twenty-four hours. The turf is low and varies from grey and sparse to whitish-grey and woolly, depending on the strain. The sporangia are moist and rhizoids and stolons are somewhat reduced. At temperatures of 57-60°C sporangia are limited or absent and growth consists of low, white, densely matted colonies, with a large proportion of subterranean mycelium. A distinct odour of urea or waste nitrogenous compounds is apparent at

these elevated temperatures.

The mycelium is hyaline, 3-6 μ in diameter, usually restricted in density. Stolons are reduced and sporangiophores arise singly or more usually in pairs from reduced nodal regions. Rhizoids vary from 60-180 μ in length and 3-5 μ in diameter. Sporangiophores are smooth, hyaline, but occasionally pale brown in old cultures, varying in length from 400 μ to over 1500 μ, 15-30 μ in diameter but up to 40 μ wide at the base. Sporangiophores usually branch frequently and mostly sympodially. The habitat view of the sporangiophores can be seen in Figure 26a. Sporangia are black, usually moist, 70-180 μ in diameter, although sporangia of smaller size may occur in dense groups in old cultures. Sporangiospores are pale brown, smooth, spherical, (2.5)3-4.5(5) μ.

Zygospores were originally formed abundantly in all the strains examined, however, subculturing has resulted in a complete absence of zygospores from the subsequent cultures. Crossing experiments have been carried out but without success, and it may be that this species is affected by continuous subculturing on artificial media. When present the zygospores occur in groups in a dense,

curly, mycelial mat surrounding the sporangiophores. Zygospores are golden brown, occasionally dark brown, smooth, spherical with conspicuous striations on the surface, 35-40(50) μ in diameter. Suspensors are swollen and short in length (see Figure 26e.-f.).

Dr. C.W. Hesseltine (1968 personal communication) agrees with my assessment that this represents a new species. Because of the reduced stolons and to a certain extent the reduced nodal region, he feels that this species may be intermediate between the genus Mucor and the genus Rhizopus. Cultures of Rhizopus sp. 1 have been sent to the United States Department of Agriculture (Agricultural research service) where they have been lyophilised and assigned the following numbers in their collection: NRRLA - 16,033 and NRRLA - 16,681.

Dr. Hesseltine has expressed his interest in high-temperature Mucorales and a publication on the true taxonomic position of this species may be forthcoming. From the temperature-growth relationships of Rhizopus sp. 1 (see Chapter IV) it is obvious that this species is strongly thermophilic and fits the definition of a true thermophile.

Figure 26

Rhizopus sp. 1

a. Habitat view of the sporangiophores in the undisturbed condition.

b. Columella.

c. Spherical sporangiospores.

d. Rhizoid development at the node.

e. Zygospore, in the immature state.

f. Mature zygospore, showing surface striations, and the swollen suspensor cells.

Figure 26

Rhizopus sp. 1

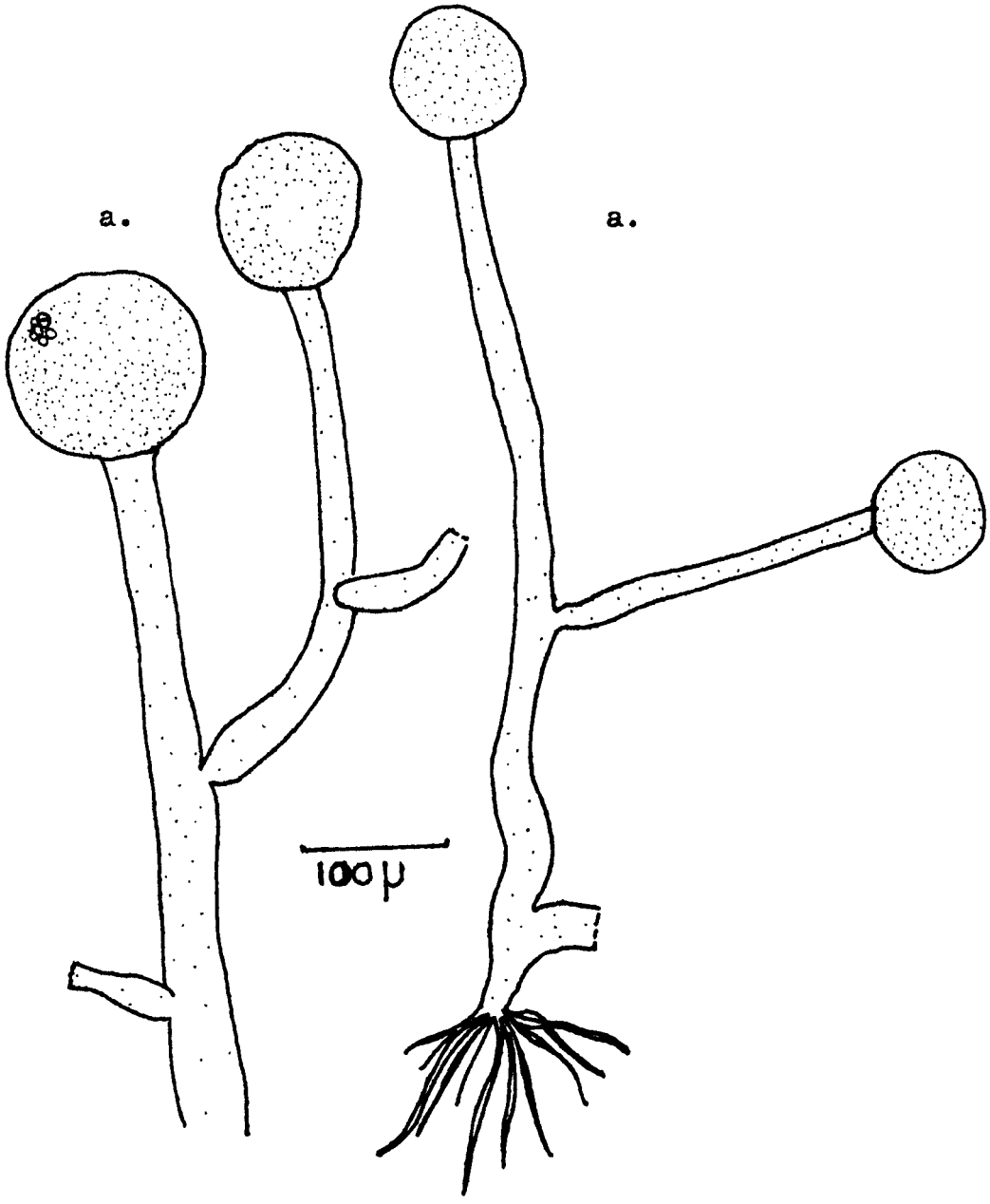


Figure 26

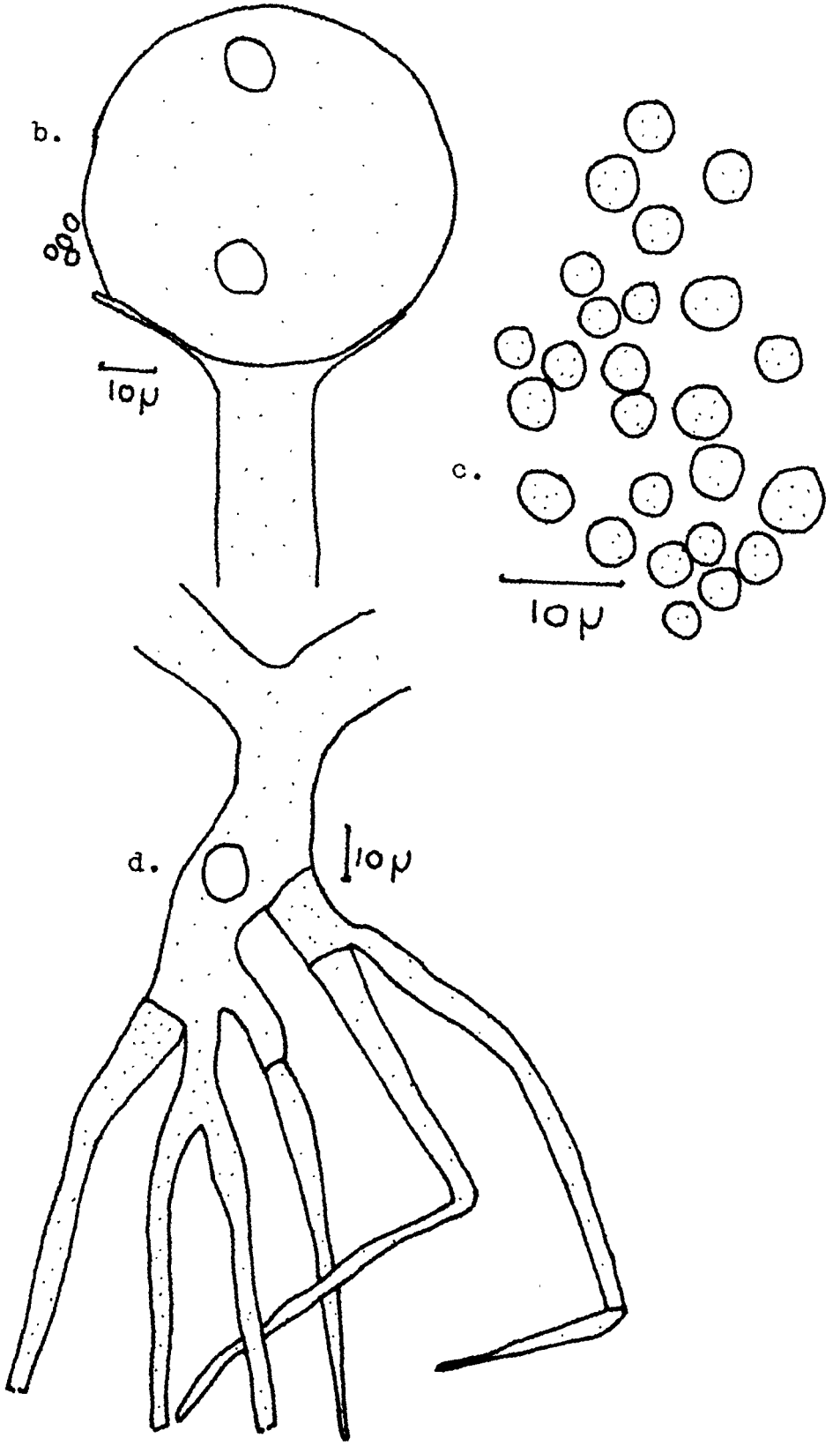
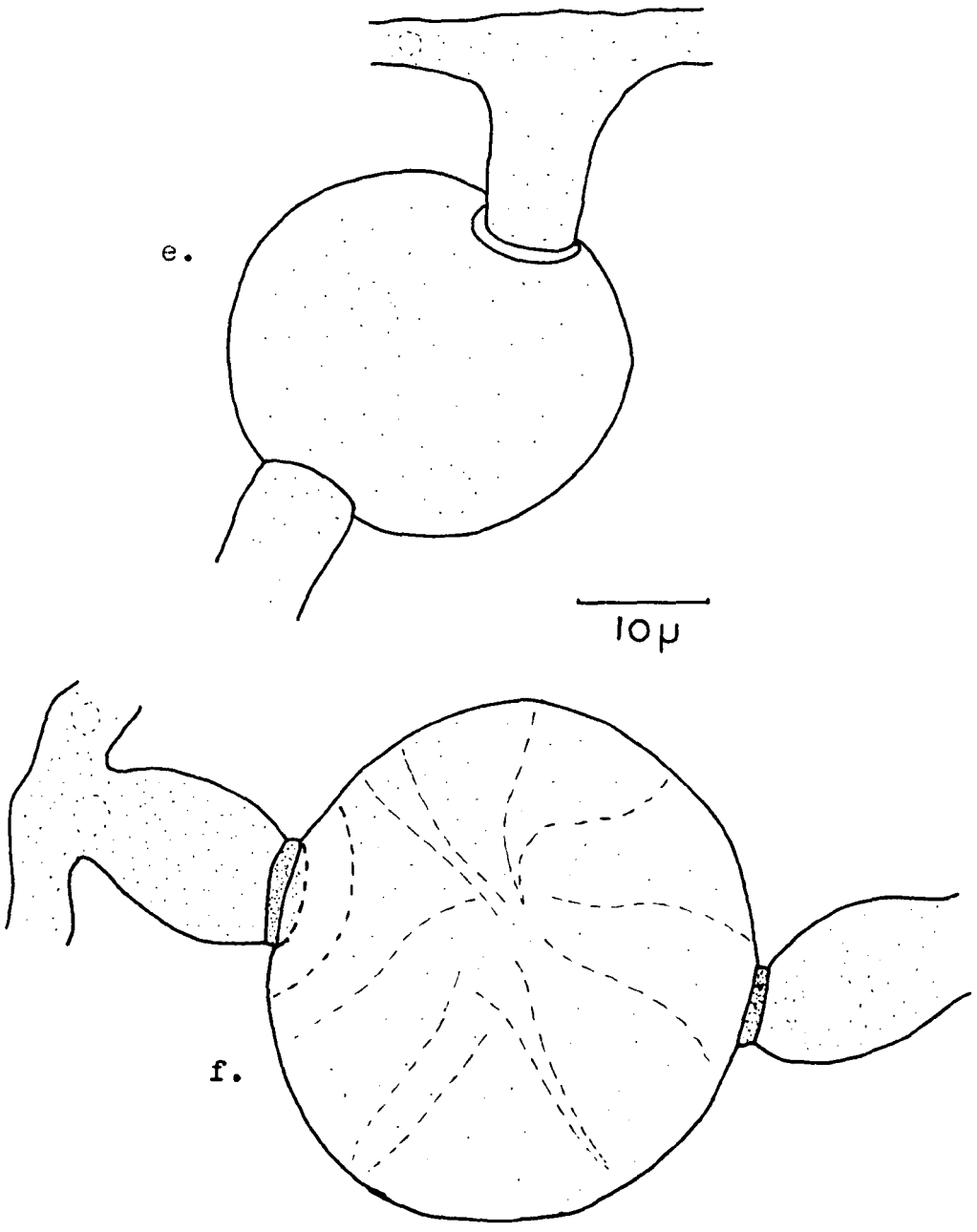


Figure 26

Rhizopus sp. 1



Rhizopus sp. 2

Identified as Rhizopus microsporus van Tieghem.

A brief description of this species appears in Les Mucorinées de la Suisse, Lendner, pp.113-115, 1908. A more detailed taxonomic study was made by Inui et al. (1965) in comparison with a number of species of the same genus.

Rhizopus sp. 2 was isolated mainly from coal spoil tips where it occurred in abundance in the warm areas, and was often the dominant species on the soil plates from these areas.

On potato dextrose agar at 40°C growth is extremely rapid and dense, floccose, white colonies are produced, although sporing remains light at this temperature. The coarse, cotton-like aerial mycelium is very characteristic at elevated temperatures but at lower temperatures the aerial mycelium is less dense and sporing is heavy, the colonies becoming light or grey-brown. At and above 45°C growth is thin and the mycelium is mainly sub-terranean and crystals are particularly abundant on the agar surface. When viewed under the binocular dissecting microscope the protoplasm of this fungus can be seen rapidly moving through the stout aerial and substrate

mycelial strands. The morphology is in agreement with that described by previous workers, the sporangiospores varying from 4-7 μ in length.

The temperature-growth relationships of R. microsporus have been studied by Weimer and Harter (1923) and more recently have been reviewed by Inui et al. (1965). The former authors found growth to occur over a temperature range 1.5-30°C, with the optimum at 25-27°C. The latter authors review the results of temperature-growth experiments for a range of Rhizopus species. For R. microsporus the range is given as 10-41°C, with only very slight growth at 41°C. In fact these authors propose that the maximum temperature for growth be used as a basis for the classification of species of the genus Rhizopus. Only two species, R. chinensis and R. pseudochinensis, are quoted as being able to grow at 45°C on a specified medium. R. microsporus is classified as showing good growth at 37°C but no growth at 45°C. Between the results of Weimer and Harter and those of Inui et al. there would appear to be wide discrepancies in the temperature range for growth, and in particular the maximum temperature for growth, of this species. This can only be accounted for by strain differences in the

isolates studied by these workers. This must also be the case for the present isolates which show good growth at 40°C and 45°C, although growth is markedly affected at higher temperatures and no growth is present at 52°C (see Chapter IV).

The dominance of R. microsporus on soil plates taken from the warm areas of certain coal spoil tips may be due to the fast growth rate of this species at the isolation temperatures employed, equivalent to those found in its natural habitat, i.e. the warm areas of coal spoil tips. Due to environmental pressures these strains may have become adapted to unusually high growth temperatures. This may account for the significant differences in the temperature-growth relationships of these strains of R. microsporus in comparison with those examined by previous workers. It may be that this is an extremely plastic organism being able to adapt to extreme environmental changes.

Rhizopus cohnii Berlese and de Toni (Rhizopus sp. 3)

Synonym: Mucor rhizopodiformis Cohn

in Sylloge fungorum 7, p.213, 1888.

This fungus was originally named Mucor rhizopodiformis by Cohn (1884) and was subsequently

changed to Rhizopus cohnii when it became established that this species could be better placed in the genus Rhizopus. The name Rhizopus rhizopodiformis (Cohn) Berlese and de Toni can be considered as a valid name for this species.

R. cohnii was isolated from a number of habitats although not in any significant amounts. It grows extremely rapidly over a wide temperature range producing white, grey and later almost sooty-black colonies, as a result of very heavy sporulation. Sporangiophores occur on the aerial mycelium as well as directly on the agar surface. Morphologically these isolates approximate those previously described.

The thermotolerant nature of this species is well documented and the initial isolates studied were obtained from lesions occurring on warm-blooded animals. R. cohnii is not described by Inui et al. (1965) and hence is not included in their classification scheme with the species capable of growing at 45°C. It would undoubtedly, however, be included in this group as the present strains were capable of growth up to 55°C (see Chapter IV).

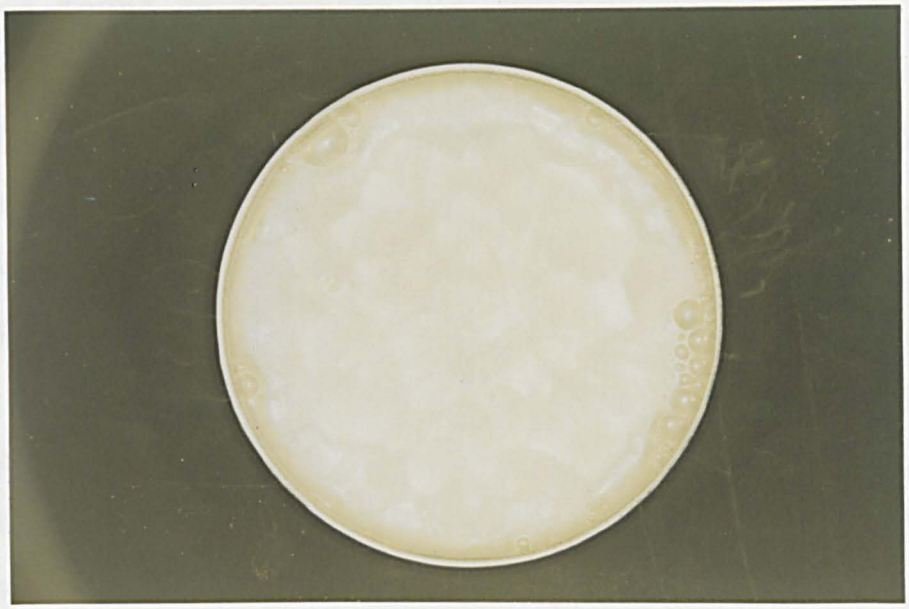
b) Mortierellaceae

Mortierella sp.

Isolated exclusively from the warm areas of several coal spoil tips. It would appear that this species has a limited distribution.

Growth at 40°C on potato dextrose agar and cornmeal agar is rapid and a cotton-like, white, aerial mycelium is profusely developed, as well as a low surface growth which produces a rosette of overlapping lobes (see Plate 8a), typically produced by many species of Mortierella. On malt agar an extremely dense, bushy, white mycelial mat is formed but the rosette arrangement is never observed because of the formation of a cream-coloured pellicle on the agar surface (see Plate 8b). At the lower temperatures (20-25°C) growth is very much slower and little or no aerial mycelium is apparent on potato dextrose agar, the typical rosette habit, however, is maintained.

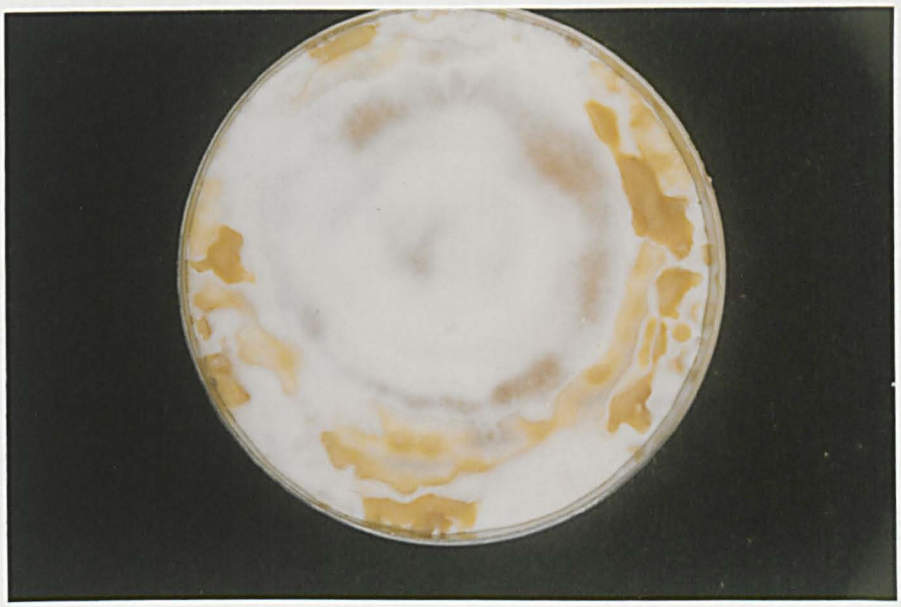
Attempts to locate mature sporangia were initially unsuccessful and the asexual stage was not adequately observed until coconut milk was included in the growth medium. Sloan et al. (1960) reported that coconut milk



a. On potato dextrose agar.

Plate 8 Mortierella wolfii: 7 days at 45°C.

b. On malt agar.



was found to increase the sporulation of a wide variety of fungi. In the present study the extracted coconut milk was filtered through several layers of cheese-cloth and autoclaved in the agar medium (50 mls./litre). The most suitable medium was found to be cornmeal agar but sporing was never abundant on any of the media used.

The sporangiophores were found to be very delicate and could never be successfully mounted on a slide without disturbing their natural state. The habitat drawings (see Figure 27a) of the sporangiophores and sporangia were made directly from the agar surface using the high power of the microscope. Sporangiophores arise mainly from the subterranean or surface mycelium but also occasionally occur on the aerial mycelium. They are very variable in length, 50-200 μ (very rarely longer), stout at the base, 4-10 μ wide, and tapering to a narrow apex, 1.5-2.5 μ wide, from which the sporangia develop. Branching may occur near the apex and two or occasionally three branches are produced (see Figure 27b). Mature sporangia are slimy, globose to spherical, light brown, the surface of which may appear slightly uneven, mostly 20-30 μ in diameter, although sporangia on the side branches may be as small as 10μ. Sporangiospores are

Figure 27

Mortierella sp.

a. Sporangiophore structure; drawn from agar medium.

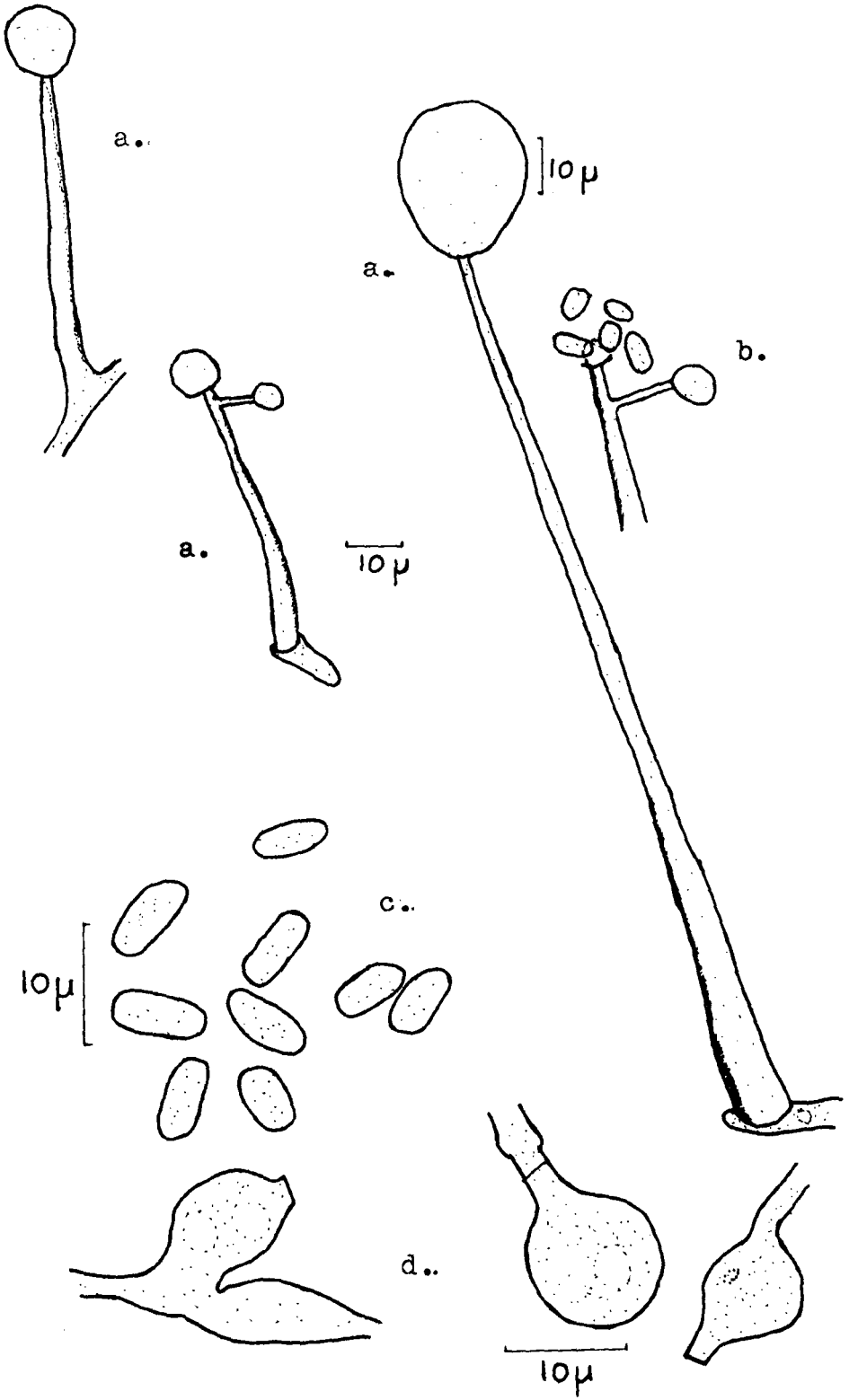
b. Dehisced sporangium showing slight columella.

c. Sporangiospores.

d. Chlamydospores.

Figure 27

Mortierella sp.



oblong to elliptical, hyaline, smooth, mostly 5-7.5(8)μ by 2.5-3.5μ. Chlamydospores are formed in and on the agar surface and take the form of single or chains of roughly spherical, swollen cells. However, the shapes of these chlamydospores may be very variable and sizes range from 8-20μ, although larger spores are occasionally found. Knots of hyphae are formed in abundance on some media but zygospores have never been observed.

Cooney and Emerson (1964, p.106) discuss the possible occurrence of a thermophilic Mortierella sp., as described by an earlier worker and they speculate on the future findings of a thermophilic, mucoraceous fungus probably belonging to the genus Mortierella. They liken the earlier species to M. turficola Ling-Young, but the present isolates fail to fit this description.

From sporangiospore shape and size the present fungus was initially likened to M. exigua Linnemann (1941, p.44) but chlamydospore shape and general sporangiophore morphology failed to compare. Finally the species was traced with the help of Dr. W. Gams (Centraalbureau voor Schimmelcultures) to M. wolfii Mehrotra and Baijal (1963, pp.52-54).

This species has been reported only from India and

the type strain, according to Dr. Gams, has been lost. Therefore, the isolates are especially interesting. It must be supposed that the type species had similar temperature relationships for growth as the present isolates (see Chapter IV) and this may explain the occurrence of this species in India where temperatures are particularly high during the summer months. Its slow growth at normal isolation temperatures may explain the rarely reported occurrence of this species and its apparent absence from temperate zones.

Ascomycetes

Allescheria terrestris Apinis

Nova Hedwigia 5, pp. 68-71, plate 3(1), Figs. A-G, 1963.

This species was frequently isolated during the present study from a number of different habitats. The fungus grows well on potato dextrose agar and yeast-starch agar at 40°C, producing dense, white, floccose colonies occasionally tinged with pink due to the production of exudate droplets. Brown cleistothecial initials appear after several days but mature cleistothecia are sparsely formed by most strains examined. However, several strains isolated from a coal spoil tip readily produce mature, dark-brown cleistothecia when grown on potato dextrose agar. The production of brown hyphae in and on the agar surface occasionally give cultures a dark-brown appearance when viewed on the reverse side of the plate. The morphological characteristics of these isolates agree well with those described by Apinis.

Aspergillus nidulans (Eidam) Winter (Emericella nidulans) in Raper and Fennell, The Genus Aspergillus, pp.495-498, 1968.

A. nidulans was isolated infrequently from coal

spoil tips and frequently from the air spora. Colonies are relatively fast-growing at 40°C on potato dextrose agar producing bright green, velvet-like growth. Development of the cleistothecia is preceeded by the production of yellow hullë cells entirely surrounding the cleistothecial initials and eventually the mature cleistothecia. In older colonies the production and subsequent liberation of ascospores results in the appearance of dark-red or purple sectors. Several strains were found to produce conidial heads only sparingly and the colonies assumed a pink and occasionally a wine red colouration. The morphology of the asexual and sexual stages correspond with those described by Raper and Fennell.

Chaetomium thermophile La Touche
Trans Br. Mycol. Soc. 33, pp.94-104, 1950.
This species was revised by Cooney and Emerson (1964, pp.62-71) who proposed three varieties, viz:

Chaetomium thermophile var. thermophile La Touche
C. thermophile var. dissitum Cooney and Emerson
C. thermophile var. coprophile Cooney and Emerson

Growth of the three varieties on potato dextrose agar at 45°C is rapid but consists mainly of a thin, ramifying, predominantly subterranean mycelium and mature

perithecia never develop. On cornmeal agar mycelial formation is still restricted but dark brown perithecia rapidly develop after several days and give the colonies a more dense appearance. However, all the varieties produce optimum growth on yeast-starch agar, and it is on this medium that the three varieties can more readily be delimited.

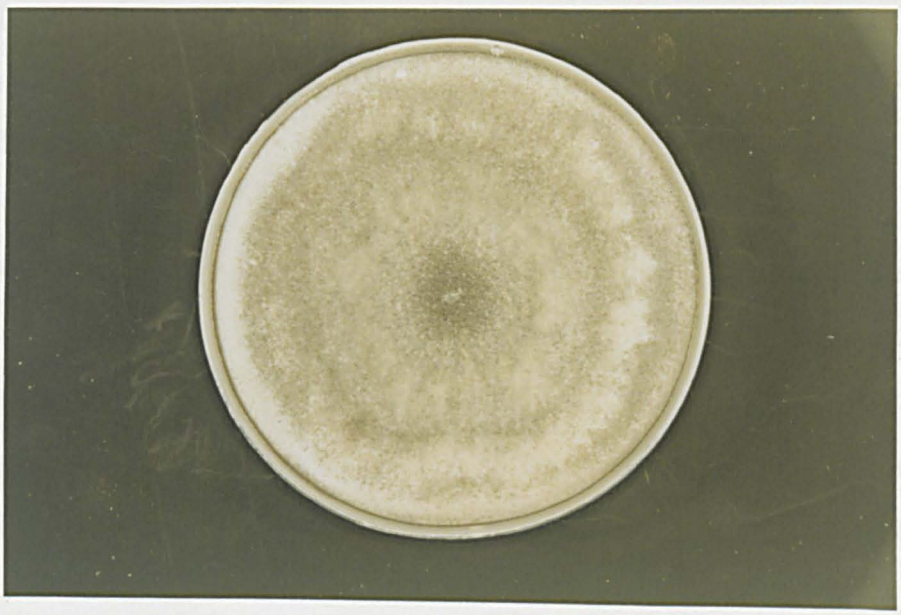
C. thermophile var. thermophile and var. dissitum both produce a relatively dense, grey turf on yeast-starch agar and perithecia are produced in varying densities on the agar surface and in the thin aerial mycelium. In the former variety perithecia are more numerous and occur in dense aggregations. In the latter variety the perithecia are less numerous and more randomly dispersed over the entire agar surface (compare a. and b. Plate 9). In both varieties the apical hairs are much better developed than the hairs covering the rest of the perithecium. C. thermophile var. coprophile produces an extremely dense growth on yeast-starch agar and the perithecia usually develop in thick concentric bands separated by grey or white areas of mycelium, producing very distinctive colonies (see Plate 10a).

The entire perithecia are covered by dichotomously-branching hairs giving them a woolly appearance which

a. var. dissitum.

Plate 9 Chaetomium thermophile
7 days on yeast starch agar at 45°C.

b. var. thermophile.



readily distinguishes them from the perithecia produced by the other two varieties. Asci and ascospores of all three varieties are similar and as described by Cooney and Emerson. Type strains of all three varieties have been examined at the Commonwealth Mycological Institute.

The morphological similarities between the varieties thermophile and dissitum make them difficult to separate and it is only the relative densities of perithecial production which denote these two varieties, and it is considered that insufficient or insignificant differences exist on which to justify their varietal status. The classification was adhered to during isolation and the three varieties were delimited depending on their growth patterns on yeast-starch agar.

Chaetomium sp. 1

This was isolated infrequently from a coal spoil tip and from the air spora.

Growth on potato dextrose agar at 40°C is rapid and compact, dark-green almost black colonies are formed with a black reverse. On cornmeal agar thin, grey to dark-brown colonies are produced. Perithecial initials are formed on all media but perithecia develop irregularly. At slightly lower temperatures perithecial production

is improved but mature perithecia never occur in any abundance. Cornmeal agar plus coconut milk was found to be the best medium for sporulation. At 20°C growth is extremely slow and colonies are light or gingerish-brown at first, gradually darkening with age, thin and mostly submerged.

Perithecia are yellow at first becoming dark brown or black at maturity. Spherical to globose in shape with a basal tuft of root-like hairs but otherwise sparsely clothed. Mycelium consists of brown, smooth or rough, thick-walled hyphae, 2.5-5 μ in diameter. Perithecia range in size from 80-180 μ, mostly about 150 μ (see Figure 28a). Asci are sac-like (mostly globose), eight-spored, 13-17 μ by 10-14 μ. Ascospores are smooth-walled, olive-brown, one-celled, apiculate, with two germ-pores, 7.5-8.5 μ by 5-5.5 μ, irregularly arranged in the ascus.

The absence of conspicuous hairs surrounding the perithecia, the irregular opening of the latter structures and the presence of globose almost spherical asci makes this species distinct from other species of the genus Chaetomium, having more in common with species of the genus Thielavia. A key to the genus Thielavia is given

Figure 28

Chaetomium sp. 1 (*Thielavia* sp.)

a. Habitat view of cleistothecium.

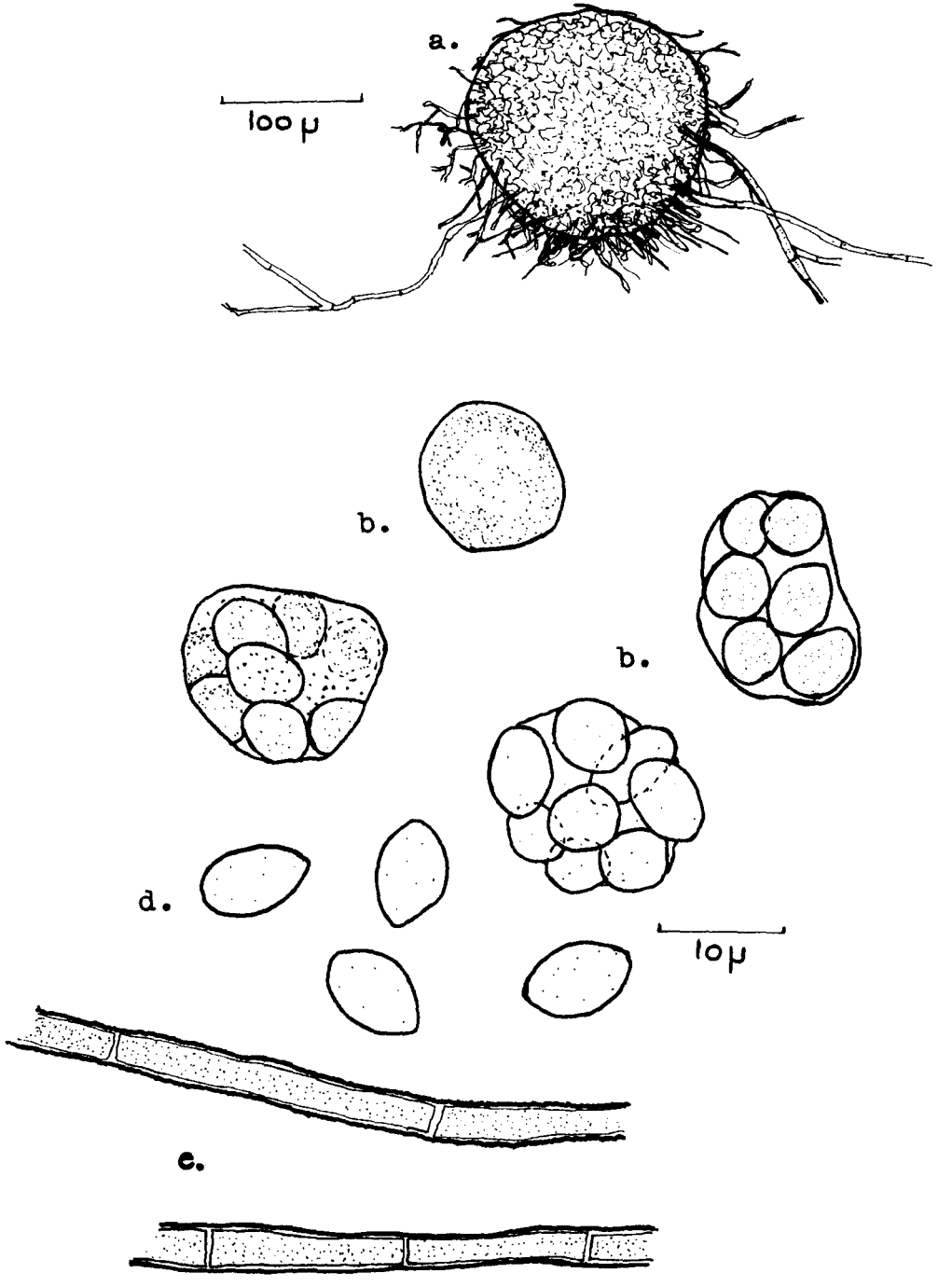
b. Stages in ascus development.

c. Rough and smooth, thick-walled hyphae.

d. Ascospores.

Figure 28

Chaetomium sp. 1 (Thielavia sp.)



by Booth and Shipton (1966) and using this key the only species which approximates the present species is Thielavia terricola var. minor, distinguished from the type species by the small ascospores (9-11μ). This variety was briefly described by Rayss and Borut (1959, p.160) and its position further elucidated by Booth (1961) in his monograph on the genus Thielavia. The present species differs, however, from this variety in several respect, viz: basal appendages on the perithecium (cleistothecium), size and shape of the ascus. Accurate classification is impossible at the moment but further study may elucidate the true position of this species.

Another species of the genus Thielavia, T. sepedonium, was isolated on a single occasion from the air spora. Growth of this species at 45°C was slow and negligible or absent at higher temperatures. An asexual stage similar to that of Thermomyces lanuginosus was present over a wide temperature range, giving the colonies a bright yellow colour. Cleistothecia were produced only at the lower temperatures, conspicuous by their dark-brown or black colouration.

It would appear, therefore, that several species of the genus Thielavia possess thermophilic properties

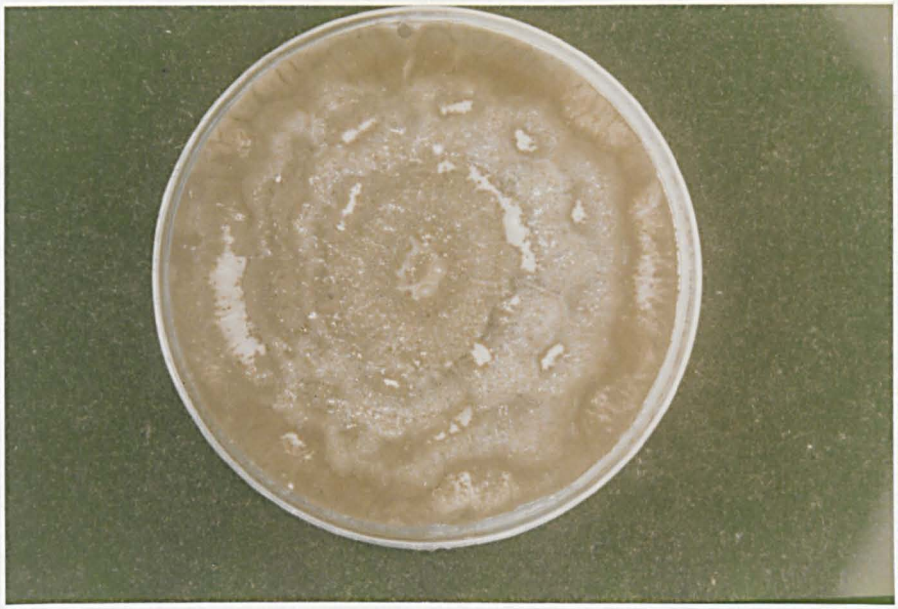
and the present isolates of Thielavia sp. (Chaetomium sp.1) show quite a marked degree of thermotolerance, being capable of growth up to 52°C (see Chapter IV).

Chaetomium sp. 2

This was isolated infrequently from a single coal spoil tip.

On potato dextrose agar at 40°C growth is very rapid and yellow bushy colonies are initially formed, similar to primrose yellow (Ridgway, p.16). Colonies gradually darken with age (see Plate 10b) as a carpet of perithecial initials is produced over the entire agar surface. The yellow initials are surrounded by light brown hairs which become pigmented imparting a ginger colour to the colony, dark brown reverse. Ginger-brown exudate droplets may also be present and on the old hyphae dense, ginger-brown, crystalline deposits are laid down on the walls (see Figure 29d) and this is partly responsible for the characteristic ginger colour of old colonies. At lower temperatures colonies remain light yellow in colour and consist primarily of a thin bushy mycelium - few perithecial initials are formed.

Although numerous perithecial initials are formed on most media, relatively few mature perithecia can



a. Chaetomium thermophile var. coprophile.
7 days on yeast-starch agar at 45°C.

Plate 10

b. Chaetomium sp. 1
7 days on potato dextrose agar at 40°.

be readily located. In situ the perithecia appear to be densely clothed in hair but when individual perithecia are separated and mounted on a slide they are observed to have a relatively sparse covering of hair but a conspicuous mat of swollen, short, brown hyphae is present at the base. Mature perithecia are globose to spherical and may be slightly elongated near the apex, (see Figure 29a) from which a cluster of brown, smooth, septate hairs arise, 2-3 μ in diameter. Perithecia measure 100-200 μ in diameter but occasionally are smaller, especially on certain media when mature perithecia are rarely found. Asci are formed in a basal tuft and when the perithecia are squashed the asci are extruded from the ostiole usually still in this arrangement. The asci are short-stalked, cylindrical, eight spored and uniserriate, 50-60 μ by 5-6 μ. Ascospores are golden-brown to olive-brown, bi-apiculate, lemon-shaped (see Figure 29c),(7.5)8-10 μ by 5.5-7 μ.

An attempt was made to trace this species in the monograph of the Chaetomiaceae (Ames 1961). Asci and ascospores approximate in size those of C. thermophile but these are the only characteristics in common. Two other species, C. brittanicum and C. virginicum were

Figure 29

Chaetomium sp. 2

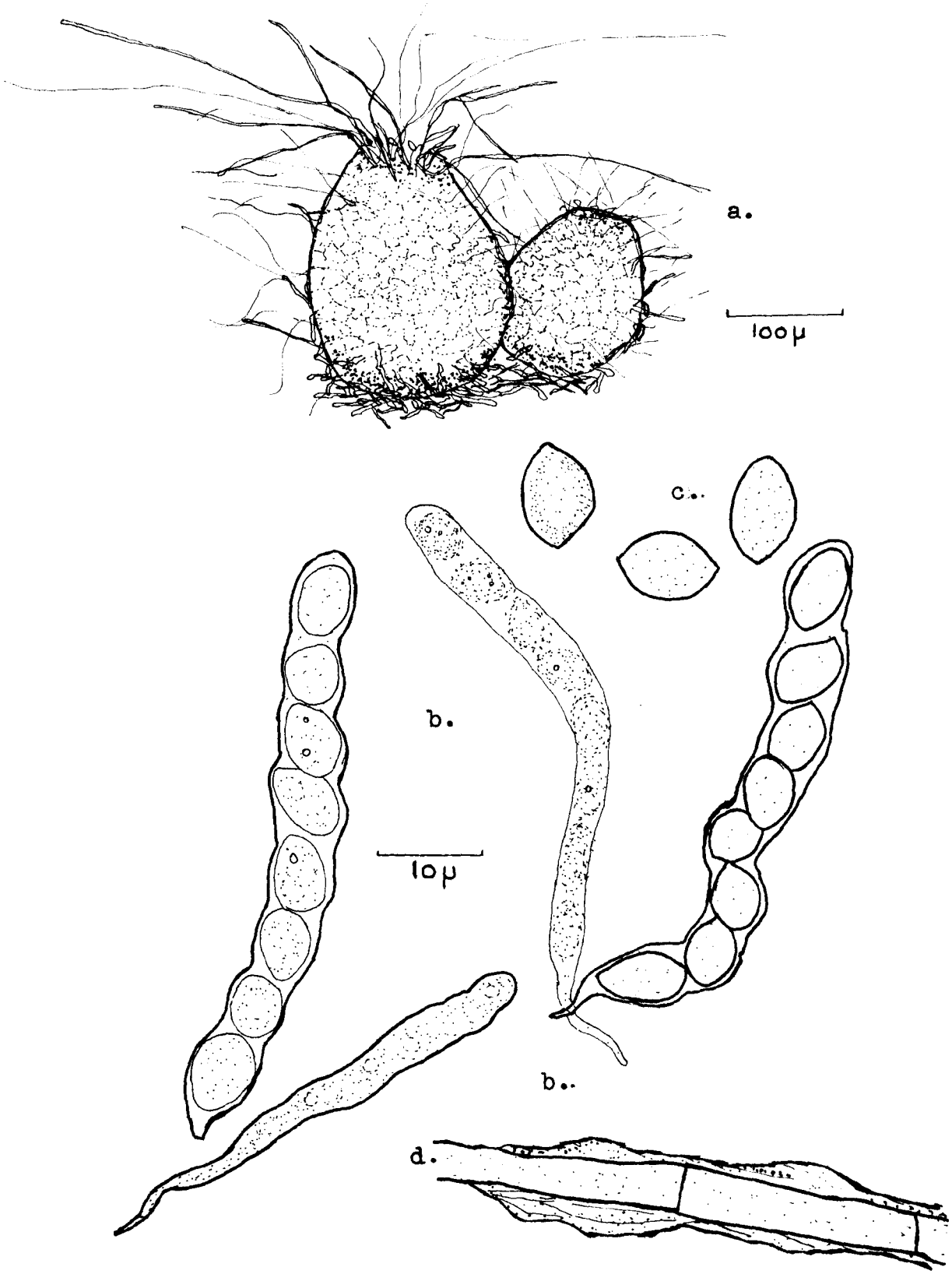
a. Habitat view of perithecia.

b. Asci, stages in development.

c. Ascospores.

d. Vegetative mycelium with crystalline deposits.

Figure 29



described by Ames as possessing thermophilic properties but they fail morphologically to agree with the isolates of Chaetomium sp. 2. It is thought that this may be an undescribed thermotolerant Chaetomium species and it is hoped to report further on its taxonomic position in the near future. Cultures have been deposited at the Centraalbureau voor Schimmelcultures, Baarn.

Dactylomyces crustaceus Apinis and Chesters
Trans. Br. Mycol. Soc. 47, 428-429 (1964).

A certain amount of confusion regarding the taxonomic position of this species has arisen over the past few years. Cooney and Emerson (1964, pp.39-50) described D. crustaceus in great detail but in fact applied the name Thermoascus aurantiacus to their species. This explains the low thermophilic tendencies of their isolates in comparison with valid strains of T. aurantiacus. Stolk (1965, pp.272-275), however, pointed out this error when a comparison of strains of the two species was made and subsequently she transferred D. crustaceus to the genus Thermoascus, on the assumption that Dactylomyces is synonymous with Thermoascus. She separated the two species on the following points: T. aurantiacus having small, elliptical ascospores and

being without a conidial stage, T. crustaceus having slightly larger, oval ascospores and with a Paecilomyces as the imperfect stage. However, Apinis (1967) re-described both these species and gave reasons for assigning them to different genera, i.e. Dactylomyces and Thermoascus.

Strains of D. crustaceus were frequently isolated during the present study and morphologically agree well with those described by Apinis and Chesters (1964) and Apinis (1967). On potato dextrose agar growth is rapid and colony colour varies slightly during production of the asexual stage, varying from pale brown to orange. The bushy Paecilomyces stage usually dies down after several days although shorter conidiophores are still produced. Ascocarps are rapidly formed being white at first (see Plate 12a), gradually turning brick-red or brown. However, above 40°C ascocarps are infrequently produced and those that are formed seldom reach maturity. The asexual stage, however, is produced up to 55°C although development at and above 50°C is relatively slow. Ascocarp production is, therefore, temperature dependent, having a much narrower range than mycelial growth.

Dactylomyces thermophilus Sopp.

Skr. Videnskselsk. Christiania Mat.-Naturv.KL

11, 35-42, 1912.

This species has been re-described by Apinis (1967, pp.578-579) following its recent isolation from wood chip piles (Bergman and Nilsson 1967) and birds' nests (Apinis and Pugh 1967), after a considerable period of absence of authentic type material. During the present study D. thermophilus was isolated only from a single forest soil and it would appear to be of infrequent occurrence. This species has not previously been isolated from soil.

At 40°C growth of the dark green conidial stage is rapid on potato dextrose agar. The colonies, however, remain relatively thin and mycelial development is restricted. After 7-10 days, brick-red to dark-red ascocarps become conspicuous, these are lacking at higher temperatures. The morphology of these isolates is similar to that described for D. thermophilus by Apinis (1967), the conidia in general ranging from 5-7.5 μ by 2-3 μ, being smaller than those originally described by Sopp. Cultures have been deposited at the Centraalbureau voor Schimmelcultures, Baarn.

Myriococcum albomyces Cooney and Emerson in Thermophilic fungi, pp. 51-61, 1964.

This fungus was originally isolated from the nesting materials of domestic chickens and reports of its occurrence have been very rare. However, the fairly widespread occurrence of M. albomyces has also been reported by Evans (1968, p.587) and since then it has been isolated from several additional sources including coal spoil tips.

On potato dextrose agar at 45°C surface growth is limiting although an extensive hyaline, subterranean mycelium is produced. Mature ascocarps never develop on this medium. Growth is much more vigorous on yeast-starch agar and mature, brown to black ascocarps develop after a ten to fourteen day incubation period. A few or an extensive mat of ascocarps develops depending on the strain and until recently mature ascospores were rarely observed. However, a strain isolated from coal spoil tips readily produces mature ascocarps in abundance with a high degree of fertility. Dark ascospores measuring 11-15 μ in diameter are found. Cultures have been deposited at the Commonwealth Mycological Institute (IMI 125,815) and at the Centraalbureau voor Schimmelcultures.

It was previously concluded (Evans 1968) that the widespread occurrence of M. albomyces may be due to the prodigous development of thick-walled hyphal cells functioning in the same manner as asexual spores, the species being dependent on its sexual stage for its survival during unfavourable conditions, i.e. low soil temperature. The scarcity of ascospore-containing ascocarps, even on natural substrates, would seem to be a limiting factor in the survival of this fungus.

Talaromyces duponti (Griffon and Maublanc) Emerson
in Raper and Thom, pp.573-577, 1949.
and Cooney and Emerson, pp.28-38, 1964.

St. conid. Penicillium duponti Griffon and Maublanc
Bull. Soc. Myco. Fr. 27, p.72, 1911.
also designated T. duponti (Griffon and Maublanc) Apinis in Nova Hedwigia 5, p.72, Plate 4(2) Figs. E-J 1963.

More recently a new epithet has been proposed due to the lack of a valid description of this species (Stolk 1965, pp.268-272). The name Talaromyces thermophilus has been proposed and this should now be accepted as the newly emended name.

On potato dextrose agar at 45°C growth is rapid and

grey, floccose colonies are formed which gradually assume shades of pink, red or green (see Plate 11a) and exudate droplets are usually abundant, the reverse varies from dark red to brown. The asexual stage is produced in profusion and morphologically agrees well with that previously described. Ascocarps have never been observed on agar. However, cultures grown on sterilised manure and oat seeds produce a grey floccose mycelium enveloping grey, spherical ascocarps, 400-1200 μ in diameter. Asci and ascospores are as described by Stolk. Conspicuous swellings in the vegetative hyphae are also apparent and these develop into a chlamydospore stage.

This species has been isolated from a number of sources, including coal spoil tips and would appear to have a widespread distribution.

Talaromyces emersonii Stolk
Antonie van Leeuwenhoek 31, 262-268, 1965.
St. conid Penicillium emersonii

This is one of the most recent thermophilic species to be described, and during the present study it proved to be an extremely ubiquitous species.

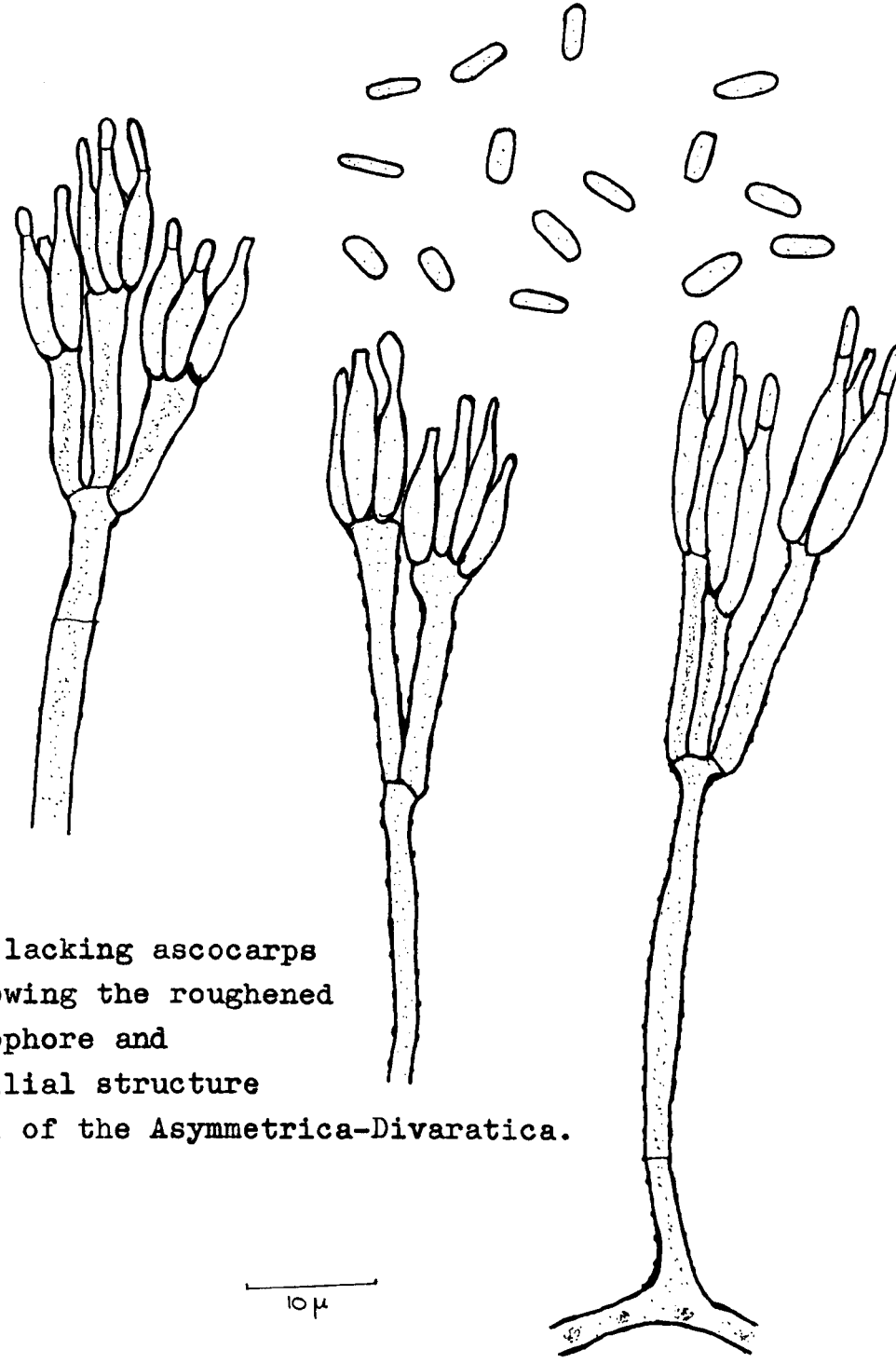
T. emersonii was found to be very variable in

culture. On potato dextrose agar at 45°C growth is extremely rapid and colonies consist initially of an asexual stage ranging in colour from pale ochraceus buff (Ridgway, plate 15) to greenish-yellow or brown. Ascocarps quickly develop and give the colonies a bright yellow or orange colouration. Pale yellow exudate droplets are occasionally produced and the colony reverse appears brown. On Czapek-Dox agar growth is slow and very much restricted, few penicilli or ascocarps are produced and a thin, subterranean mycelium results. The conidia of certain strains were found to be extremely variable in size and occasionally chains of large swollen conidia up to and over 12 μ in length were observed. Both rough and smooth-walled conidiophores have been observed.

Several unusual strains were also isolated and thought to be varieties of T. emersonii. A strain isolated from coal spoil tips (var. 1) and a strain isolated from pine-woods both failed to produce the ascocarp stage on agar. In both strains conidial production is extensive, the latter strain produces buff-coloured colonies and the former strain produces bright-yellow colonies on potato dextrose agar. This strain

(var. 1) stains the agar lime green producing a dark green reverse and in older cultures a few ascocarps have been observed, these are smaller and do not show the characteristic orange colour of T. emersonii. Dr. Stolk (personal commumication) states that this strain probably represents a reduced form of T. emersonii, perhaps a variant. Cultures have been deposited at the Centraalbureau voor Schimmelcultures, Baarn. Ascocarps have not been observed in the other strain but the structure of the penicilli and conidia (see Figure 30) agree with those described by Stolk.

Another strain (var. 2) when grown on a variety of media produces only the ascocarp stage, the asexual stage has never been observed. Growth on potato dextrose agar at 45°C is rapid and floccose, pale lemon-yellow (Ridgway plate 4) are produced, with a pale orange reverse. Exudate droplets are conspicuous and ascocarps quickly develop forming a continuous layer on the agar surface. Ascocarps, asci and ascospores (see Figure 31) are morphologically similar to those described for T. emersonii, although occasional slight roughening of the ascospore surface is evident. Conspicuous swollen cells (see Figure 31b) surrounding the ascocarps are a



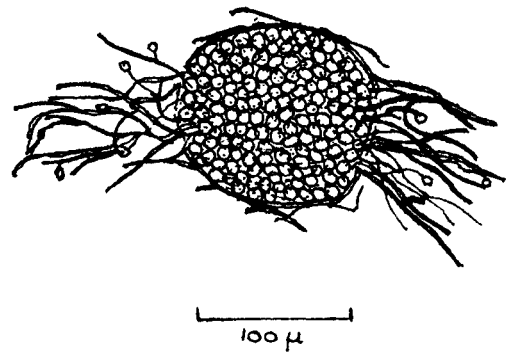
Strain lacking ascocarps but showing the roughened conidiophore and penicillial structure typical of the Asymmetrica-Divaratica.

Figure 30

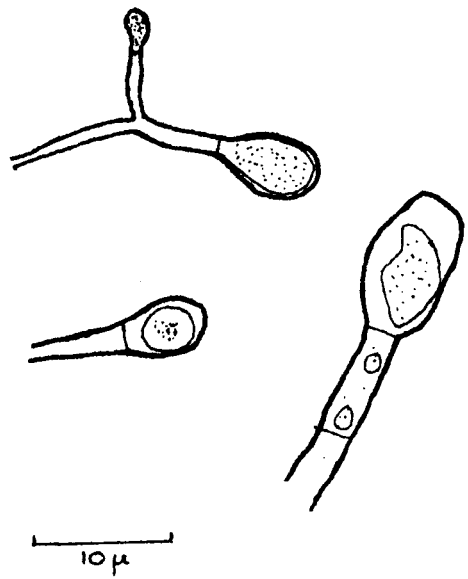
Penicillium emersonii (var. 1)

Figure 31

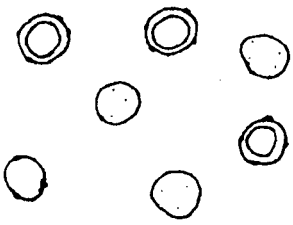
Talaromyces emersonii (var. 2)



a. Ascocarp habit

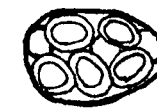


b. Swollen cells surrounding the ascocarp.



d. Ascospores.

c. Asci



feature of this strain. They have also been observed by Stolk as occurring in her strains but not so densely produced. The temperature-growth relationships of this strain (var. 2) have been compared with those of normal strains of T. emersonii (see Chapter IV) and found to differ in several respects. This strain can also be considered as a variant of T. emersonii.

Talaromyces sp.

This was isolated numerous times from a single coal spoil tip, and in particular from the lower soil horizons.

Growth at 40°C on potato dextrose agar, yeast-starch agar and malt agar is extremely rapid and colonies are compact, velvet-like with a loose aerial mycelium, margin regular in outline. The asexual stage is abundantly produced on the aerial mycelium and directly on the agar surface. Colonies initially pale ochraceus buff (Ridgway plate 15) but rapidly become yellow with the development of ascocarps, older colonies approximate straw yellow (Ridgway, plate 16). A yellow diffusate is apparent after several days and this changes to light brown and finally dark brown (see plate 11b), and colonies have a dark brown reverse. On Czapek-Dox agar growth is much

a. Talaromyces duponti
7 days on malt agar at 45°C.

Plate 11

b. Talaromyces sp.
7 days on potato dextrose agar at 45°C.

slower and thin, light yellow colonies develop, both asexual and sexual stages are present and the agar around the colonies is stained with a light yellow diffusate.

Conidiophores are variable in length, ranging from 80-400 μ and side branches may arise from the main conidiophore axis (see Figure 32d). It is generally smooth, but yellow to brown depositions have been occasionally observed in or on the conidiophore wall (see Figure 32a). Conidiophores are septate, yellow to pale brown in colour, 1.5-3.5 μ wide. Penicilli are of the Asymmetrica-Divaricata type but tend to be more compact than the majority of examples of this type. Although most of the penicilli are of the regular biverticillate type these structures may vary and occasionally are monoverticillate bearing 3-5 phialides at the apex of a short conidiophore (see Figure 32b). Rami when present measure 10-15 μ by 2.5-3.5μ. Metulae are smooth, 2-4 in a verticil, measuring 8-12 μ by 2-3μ, but they may be enlarged at the apex, up to 4 μ. Phialides occur in small clusters and may or may not be divergent, consisting of a slightly swollen base, tapering to a fairly long distinct neck region, i.e. of the P. janthinellum type.

They range in length from 10-18 μ but average about 12-14 μ by 1.5-2.5 μ. Conidia are smooth, hyaline, ellipsoid to cylindrical, 3-5 μ by 1-1.7 μ, usually formed in long tangled chains. In the vegetative mycelium swollen cells have been infrequently found and these may function as chlamydospores.

Ascocarps are formed abundantly on all media after several days growth and usually mature in seven to ten days. Initially they are white quickly turning bright yellow, thin-walled, and surrounded by numerous light-yellow branching hairs (1-1.5 μ in diameter) giving the ascocarps a woolly appearance (see Figure 32e). With the development of short, swollen, brown hyphae (1.5-3 μ in diameter) at the base the ascocarps may darken in colour. The roughly spherical ascocarps range in size from 50-250 μ but the average is about 100-150 μ. Asci are produced in helicoidal, curved chains and are globose to sub-globose, eight-spored, 6-8 μ in diameter. Ascospores are lenticular, ornated by 2-3 irregular ridges and spines (see Figure 32h). They appear yellow or orange in mass and measure 3-4 μ by 2-3 μ.

This species was initially thought to be a variant of Talaromyces (Penicillium) duponti, distinguished by

Figure 32

Talaromyces sp.

Asexual stage

a. Penicilli, variation in form but characteristic of the Asymmetrica-Divaratica series.

b. Reduced penicillus.

c. Conidia.

d. Conidiophore habit, very long conidiophores showing branching.

Sexual stage

e. Ascocarp habit.

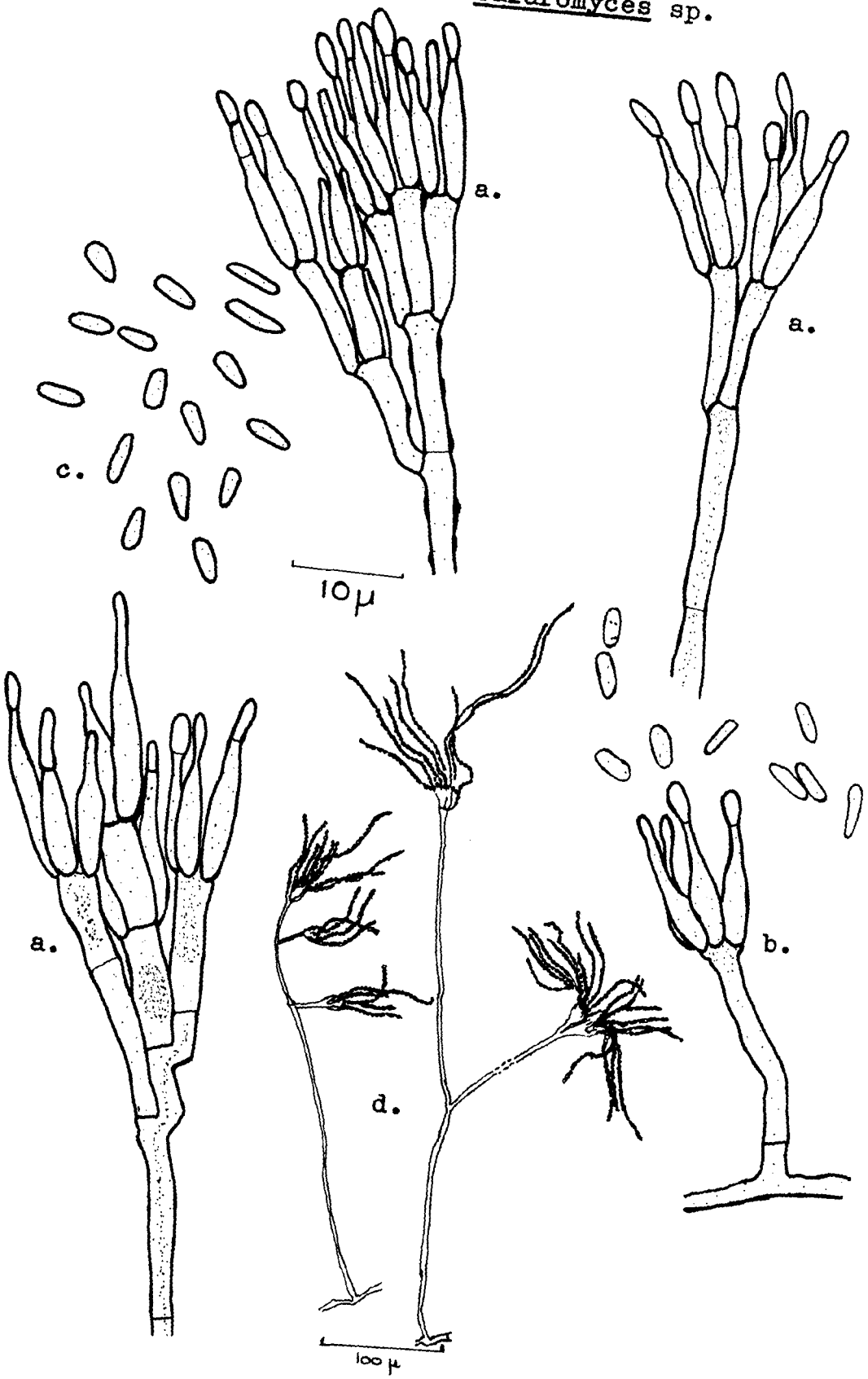
f. Swollen hyphae at the base of the ascocarp.

g. Asci, formed in curved, helicoidal chains.

h. Ascospores with spines and ridges.

Figure 2c

Talaromyces sp.



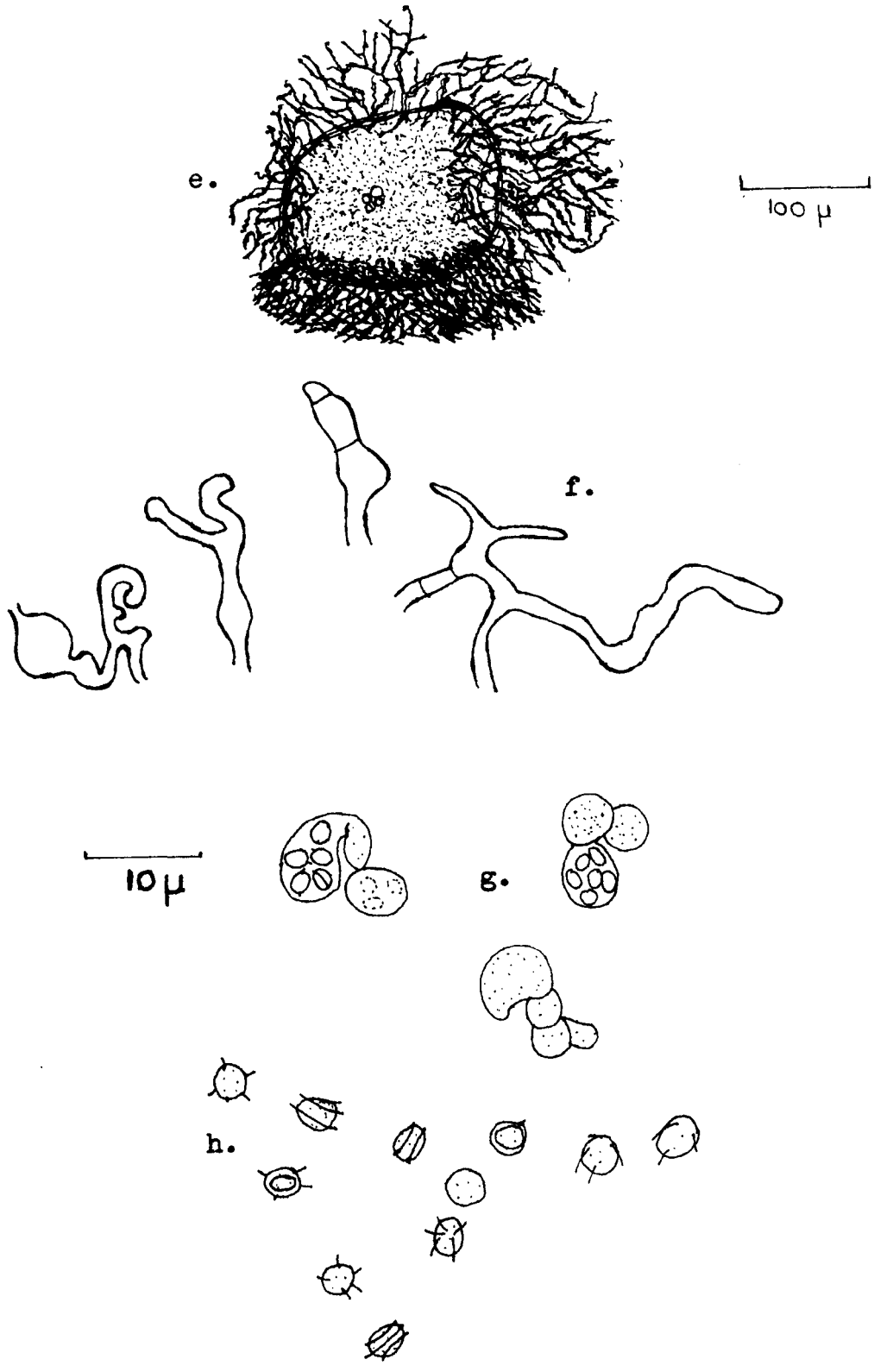


Figure 32

Talaromyces sp.

its ability to freely produce ascocarps on agar. However after a careful discussion with Dr. A.C. Stolk, Centraalbureau voor Schimmelcultures, it was finally decided that these strains were significantly different to justify their separation from T. duponti on a species level. Below is a comparative list of the differences which are considered to be significant in order to separate Talaromyces sp. from T. duponti (thermophilus).

That these two species are closely related is evident and this stems from the similar ascus and ascospore morphology. Also the penicilli are both classified in the Asymmetrica-Divaricata series, although those of Talaromyces sp. tend to be more complex and less divergent than those of T. duponti (thermophilus). Phialides are both of the P. janthinellum type with fairly long conidum-bearing tips. They can most readily be separated:

a) on the basis of their temperature-growth relationships, Talaromyces sp. being classified as a thermotolerant species and T. duponti as a true thermophile;

b) on their ascocarp morphology;

c) on their cultural characteristics.

Characteristics	*Talaromyces* sp. strains from Leycett coal spoil tip	*T. duponti* (*thermophilus*) Type strain CBS 236.58
Temp. relations °C.	min. 18 opt. 42 max. 55	min. 30 opt. 45-50 max. 60
Colony colour (on malt agar and potato dextrose agar)	Creamish buff to yellow, reverse brown (see plate 11b)	Shades of grey from pink to green, reverse uncoloured (see plate 11a)
Sexual stage	Ascocarps develop on a variety of agar media. Yellow (brown) thin-walled 50-250 μ in diameter. Asci in curved, helicoidal chains, 6-8 μ. Ascospores 3-4 μ x 2-3 μ.	Ascocarps not formed on agar, only on sterilised oats etc. Grey, thick-walled 400-1200 μ in diameter. Asci in slightly curved chains, 9-10 μ. Ascospores 3.5-5 μ x 2.5-3.5 μ.
Asexual stage	Penicilli divaricate regular. Conidiophores usually long. Conidia; ellipsoid to cylindrical 3-5 μ x 1-1.7 μ.	Penicilli divaricate, somewhat divergent. Conidiophores usually short lateral branches. Conidia; ovoid to ellipsoid. 2.5-5 μ x 1.5-3 μ.

It is hoped that a description of this species will be published with Dr. A.C. Stolk and the name Talaromyces leycetti is provisionally proposed. This is named after the area of isolation, up till now the only known source of this interesting fungus.

Thermoascus aurantiacus Miehe
re-described by Apinis, Trans. Br. Mycol. Soc. 50, pp.574-576, 1967.

As mentioned previously, T. aurantiacus has been confused with Dactylomyces crustaceus (see Cooney and Emerson 1964, pp.39-50) and has consequently been misidentified by several workers. Pore and Larsh (1967) described T. aurantiacus as being responsible for animal and human mycotic infections. Sample cultures labelled T. aurantiacus were received from the latter authors and these isolates turned out to be D. crustaceus.

On potato dextrose agar at 45°C growth of T. aurantiacus is extremely rapid and a low, white mycelial mat is initially formed, giving rise to white, tree-like mycelial strands. The white ascocarp initials develop after several days and quickly darken in colour, mature ascocarps being irregular in shape, brown or

a. Dactylomyces crustaceus.
7 days on potato dextrose agar at 40°C.

Plate 12

b. Thermoascus aurantiacus
7 days on yeast-starch agar at 45°C.

khaki-coloured, usually surrounded by a weft of thin, white hyphae. Growth on yeast-starch agar is also rapid but the ascocarps form an almost continuous, orange brown, wrinkled carpet on the agar surface (see Plate 12b). Morphologically the strains isolated during the present study are similar to those described by Apinis (1967). Confusion can arise regarding the conidial stage which may initially be overlooked, the conidia were originally mistaken for chlamydospores (terminal). One strain isolated from a pine-wood soil produced long, erect ascocarps clothed in a dense, white mycelial mat, and mature ascocarps rarely developed. Strains of this species were isolated from a wide variety of habitats and it would appear to have a comparatively wide distribution.

Trichophaea sp. (Discomycetes)
Genus Trichophaea Boudier
revised by Kanouse, Mycologia 50, 121-140, 1958.
Genus Sphaerospora Saccardo.
summarised by Cain and Hastings, Can. J. Bot. 34, 360-376, 1956.

It was thought necessary to include a discussion of these two genera of Discomycetes due to a Botrytis-like

asexual stage which occurs in two species of these closely related genera. The two genera are separated on ascospore shape, Trichophaea having ellipsoidal spores and Sphaerospora having globose or spherical spores.

Trichophaea sp. was isolated exclusively from coal spoil tips, specifically from the warm areas, and was originally thought to be a Botrytis species on the basis of its conidial stage. However, the presence of sexual bodies in older cultures was detected and the species was transferred to the genus Trichophaea. A species belonging to the genus Trichophaea and which produces a Botrytis-like asexual stage has been reported by several workers (Kevorkian 1932, Kanouse 1958, Webster et al. 1964), viz: Trichophaea abundans (Karsten) Boudier. A critical comparison of the taxonomy of T. abundans and the present isolates labelled Trichophaea sp. led to the conclusion that they were different species. An extensive search of the literature revealed another Discomycete with a Botrytis-like stage closely related to the genus Trichophaea. This was Sphaerospora minuta Cain and Hastings and the taxonomy of this species was critically compared with that of T. abundans (see Cain and Hastings, 1959).

Growth on potato dextrose agar, yeast-starch agar and malt agar is rapid at 40°C producing dense, floccose colonies, light brown to buff-coloured at first becoming dark brown in older cultures (see Plate 13a). Sporing is very heavy and colonies are brown to dark brown reverse. At lower temperatures (20°C) growth is still relatively rapid but colonies are thin and slightly floccose with only light sporing.

Conidiophores are long, mostly 150-500 μ by 5-10 μ in diameter, occasionally up to 1000 μ in length, septate, light brown, smooth, forming a loose aerial layer which may become extremely dense, especially on yeast-starch agar. Conidiophores develop directly from the agar surface or less frequently from the aerial mycelium. In old conidiophores the walls become thickened and dark brown, and occasionally conspicuous brown deposits are laid down in the walls (see Figure 33c). The conidiophores branch dichotomously, at right angles, near the apex and this may be repeated several times (see Figure 33d). The primary branches are about 50-100 μ long, the secondary branches somewhat shorter, about 30-50 μ, and the tertiary branches only about 10-20 μ long. The end of each branch is swollen to form a

globose ampulla approximately 10 μ in diameter. Each ampulla produces simultaneously a series of denticles about 1.5-3 μ long and approximately 1 μ wide. From these denticles globose conidia develop which when mature break off, often leaving the denticle as a spine on the ampulla. The conidia are smooth, light-brown, with a definite apiculate region, 4-7 μ in diameter. They appear ochraceus to brown in mass. Conidial formation and conidiophore morphology are similar to that described for Trichophaea abundans (Kevorkian 1932) and for Sphaerospora minuta (Cain and Hastings 1956). However, the methods of denticle formation and the structure of the ampullae corresponds more closely with the description of the asexual stage of T. abundans than with S. minuta. (See Figure 33a for the development of the asexual stage).

The apothecia develop after several weeks at 20-40°C and on a variety of media. They are usually borne directly on the aerial mycelium but may also be found on the agar surface. Apothecia are fleshy, light-brown to dark brown, globose to spherical, (100)150-400 μ in diameter and sessile. The excipular cells are basically regular, from which project numerous brown

Figure 33

Sphaerospora (Trichophaea) sp.

Botrytis-like stage

a. Ampullae, showing their development and the formation of conidia on denticles.

b. Conidia; apiculate.

c. Old conidiophore with deposits in the wall.

d. Conidiophore habit.

Sexual stage

e. Mature apothecium with spike-like hairs.

f. Cells of the excipulus.

g. Excipular hairs with swollen base and tubercles.

h. Immature and mature asci.

i. Uni-guttulate, spherical ascospores.

Figure 33

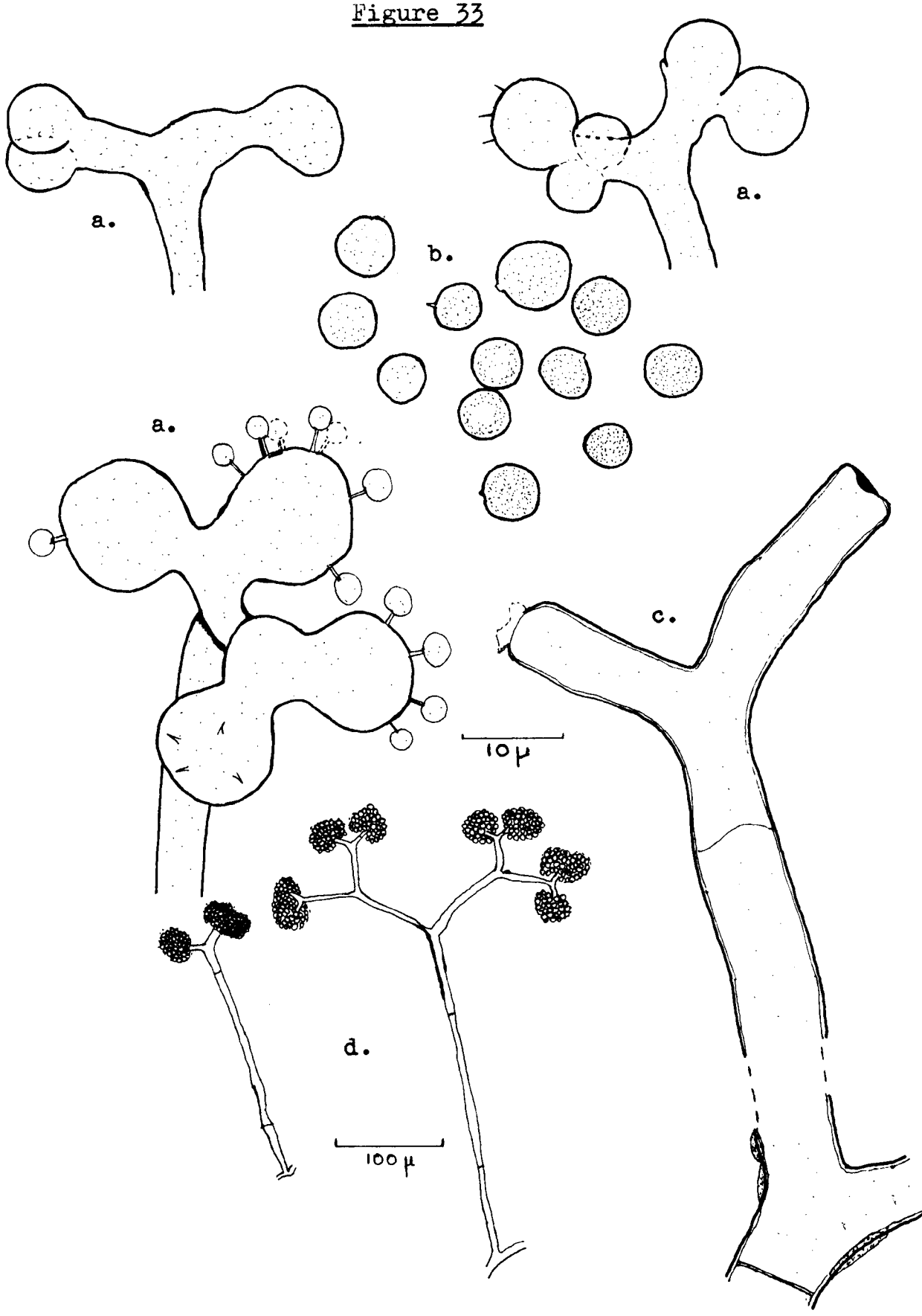


Figure 33

Sphaerospora (Trichophaea) sp.

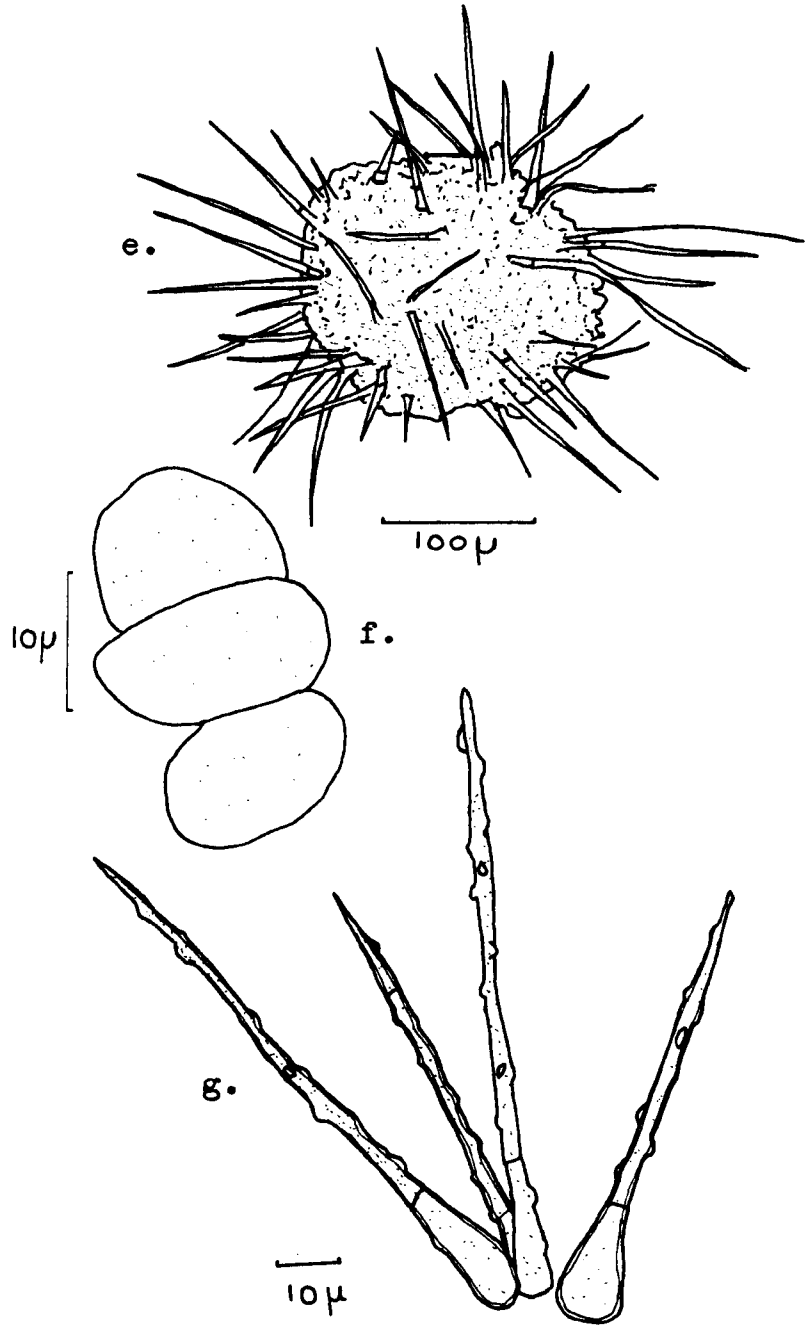
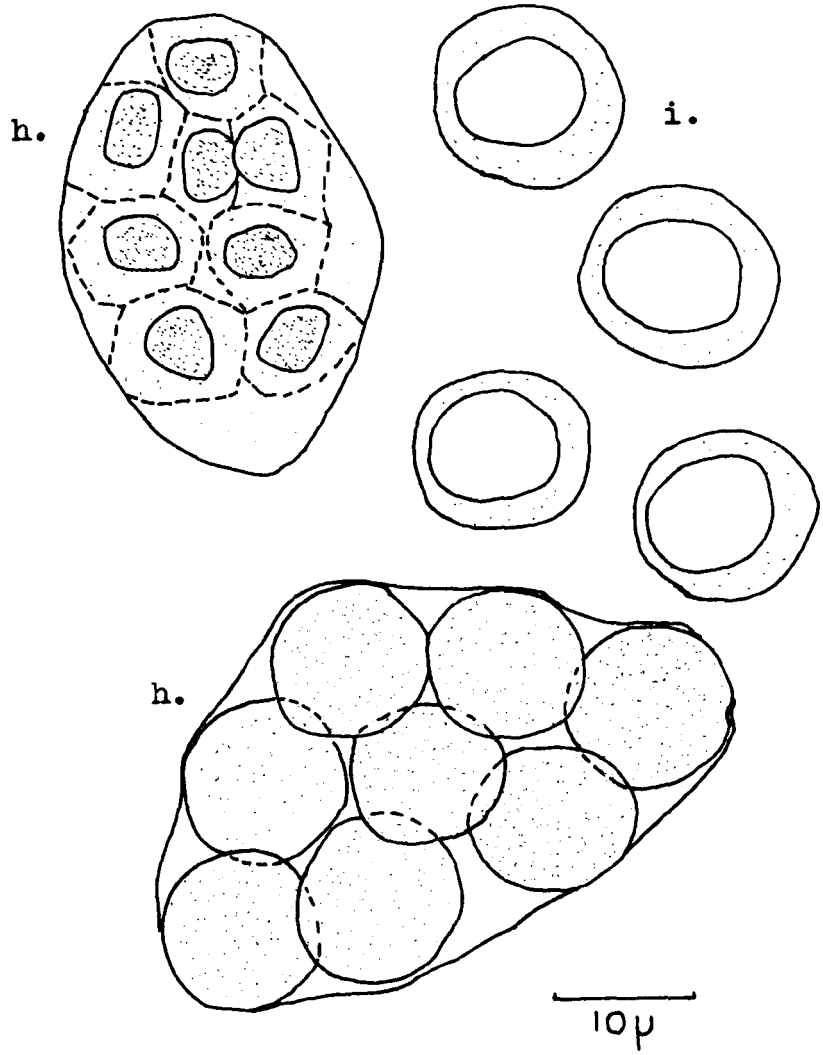


Figure 33

Sphaerospora (Trichophaea) sp.

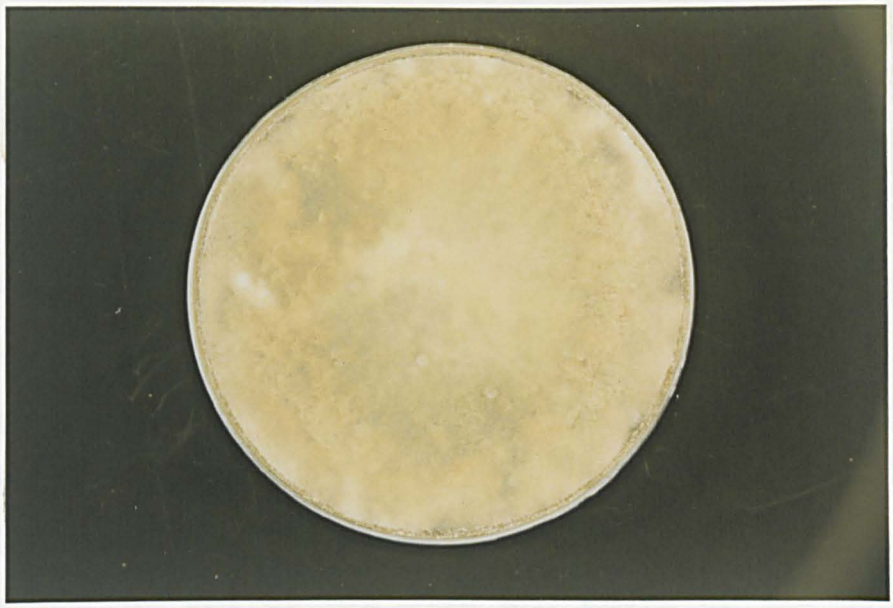


hairs. These are straight, unbranched, septate (one or two), rigid, and may be blunt or more usually pointed at the apex, 50-150 μ long and 5-8 μ wide near the swollen basal cell and tapering to 2.5-3 μ near the apex. The hairs are usually thick-walled with scattered, projecting tubercules (see Figure 33g). The cells of the apothecial wall are globose or sub-globose, approximately 8-12 μ in diameter.

Asci are sac-like or oval, and may be broadly rounded at the apex, 40-50(55) μ by 20-30 μ, eight-spored. A short stipe is occasionally visible on the asci but this is not normally observed. Paraphyses are intermixed with the asci and may be branched or unbranched. Ascospores are not definitely arranged in the ascus, although they may sometimes appear biseriate, globose, smooth, hyaline, 11-15 μ in diameter, and with a single, large, refractive oil globule (approximately 8-10 μ). Using phase-contrast microscopy the ascospores can be seen to be thick-walled.

In view of the globose ascospores this species can be more accurately placed in the genus Sphaerospora but a comparison of the three species is considered necessary because of their close morphological interrelationship (see Table overleaf).

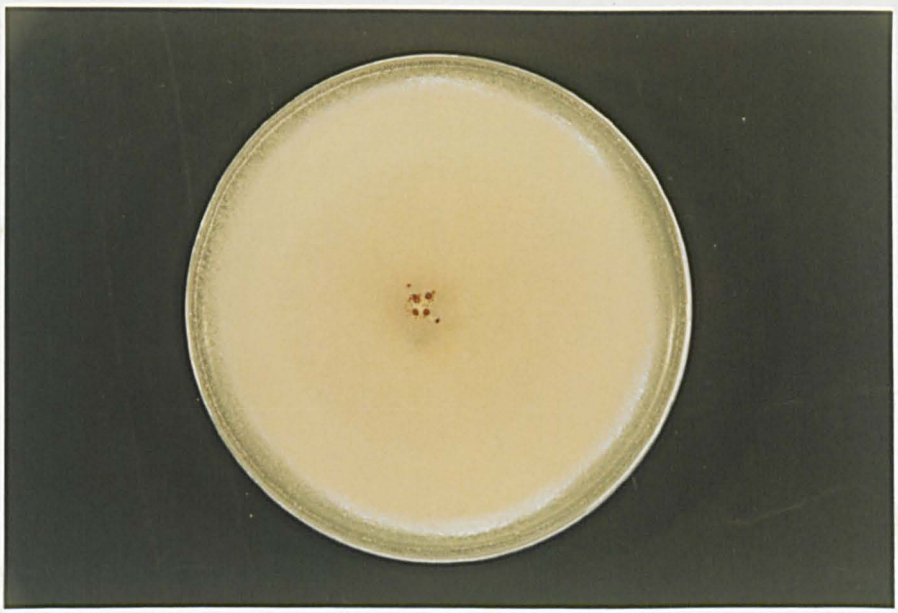
Characteristics	*Trichophaea* sp. (*Sphaerospora*)	*S. minuta*	*T. abundans*
Ampullae	Not in chains, numerous conidia per ampulla.	In short branching chains. Few conidia per ampulla.	Not in chains, 10-12 conidia per ampulla.
Conidial size	4-7 μ	6-14 μ mostly 9-10 μ	7-9 μ
Apothecia	Globose	Globose to sub-globose	Flat
Size	150-400 μ	300-1000 μ	1-3 mm.
Colour	Light brown to dark brown	Light brown	Greyish-white
Hairs	Tubercules present	Tubercules present	Smooth
Asci	Sac-like 40-55μ x 20-30 μ	Cylindrical 125-160μ x 16-19μ	Cylindrical 120-150μ x 8-10μ
Ascospores	Globose 11-15 μ One large oil drop.	Globose 13-17 μ One large oil drop	Elliptical 14-15μ x 6-8 μ Small oil drop near each end.
Temperature-growth relationships	10-50°C range 35-42°C optimum	Unknown, but said to favour summer temperatures i.e. 85°F	Approx. 10-37°C Optimum 30°C. (see El-Abyad and Webster 1968a. pp.356-357)



a. Sphaerospora (Trichophaea) sp.
4 days on potato dextrose agar at 40°C.

Plate 13

b. Aspergillus fumigatus, orange var.
7 days on Czapek-Dox agar at 40°C.



The Botrytis-like stage of all three species would appear to be very similar and only slight differences exist regarding conidial size. The apothecia differ greatly in size and significant differences would appear to exist in the morphology of the asci and ascospores of the three species. The present isolates labelled Trichophaea sp. must be placed in the genus Sphaerospora and are closely related to the species S. minuta from which they are distinguished primarily by conidial size and formation and by the size and shape of the asci. Due to the lack of information concerning the temperature-growth relationships of S. minuta, comparisons cannot be drawn. But the present Sphaerospora sp. is a thermotolerant species with a high maximum temperature for growth and because of the absence of any further species in this genus which posses a Botrytis-like asexual stage it is thought that this may represent a new species. The name Sphaerospora thermophila is tentatively proposed, a new thermotolerant Discomycete.

Fungi Imperfecti (Deuteromyces)

Aspergillus fumigatus Fresenius

Thom and Raper in Manual of the Aspergilli, pp.148-151, 1945.

Raper and Fennell in the Genus Aspergillus, pp.242-246, 1965.

The variation in strains of A. fumigatus is considerable and accounts for the extensive list of synonyms compiled by Raper and Fennell. Numerous strains or varieties were isolated during the present investigation and these constituted mainly variation in colony colour, i.e. olive, orange and brown strains were regularly isolated. Segregation of an orange mutant sector in a culture of a green strain of A. fumigatus can be seen in Plate 23b; these mutants may arise spontaneously in the laboratory but they also occurred directly on the soil plates and remained stable during continuous subculturing. The orange strain or variety is particularly distinct and approximates orange-cinnamon (Ridgway, pl.2a); this strain often produces brown exudate droplets (see Plate 13b). Also a variety of green shades was encountered, ranging from light-green, grey-green to dark green. Growth on all media is extremely rapid over a wide range of

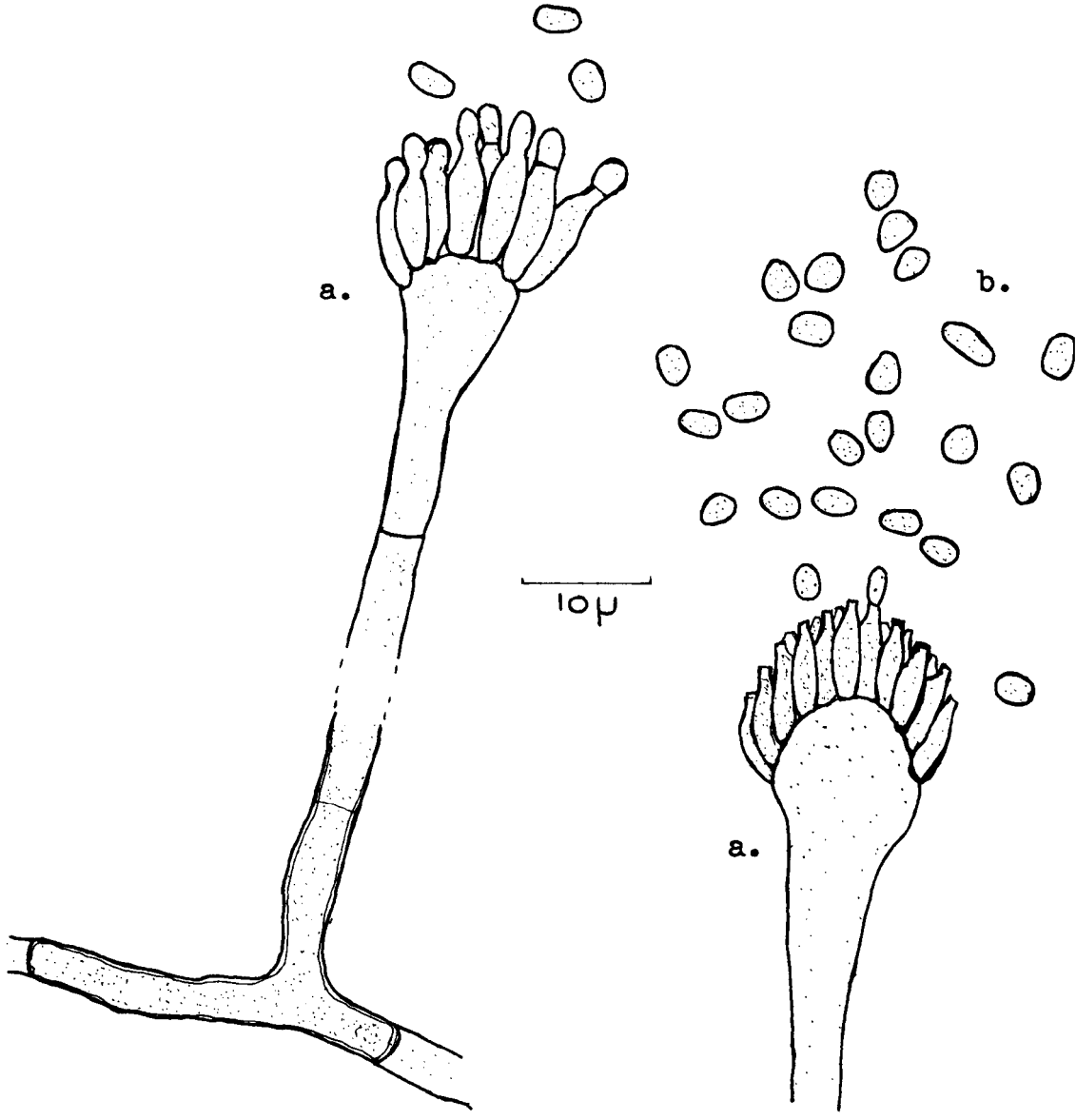


Figure 34

Aspergillus fumigatus var. ellipticus

a. Young and old conidiophores typical of A. fumigatus

b. Conidia; smooth, elliptical.

temperature and the very heavy spore production is responsible for the high incidence of contamination caused by this fungus in the laboratory. Variation in the amount of aerial mycelium is also common and in some strains little or no vegetative mycelium is present.

An unusual variety of A. fumigatus was isolated from the air spora and is distinguishable from the normal strains by the production of smooth, elliptical conidia, (see Figure 34). This was later traced to A. fumigatus var. ellipticus Raper and Fennell and the latter variety has previously only been isolated from a human mycotic infection.

Aspergillus sp. 1

Isolated from a single coal spoil tip and identified as Aspergillus fischeri Wehmer.

Raper and Fennell, The Genus Aspergillus, pp.252-255, 1965

Ascosporic state reported as:

Sartorya fumigata Vuillemin fide Benjamin. Mycologia 47, 678, 1955.

Rapid growth on potato dextrose agar at 40°C, a cotton-like aerial mycelium is formed and a thin, white substrate mycelium is present which gradually turns pale

yellow. Conidial heads are sparingly produced at this temperature, and indeed at most temperatures. Cleistothecial initials in the form of knots of white hyphae are formed but never develop any further at 40°C. Cultures incubated at 30°C produce cleistothecia in abundance and the colonies may become extremely floccose. The morphology of the asexual and sexual stages agrees well with those reported by the above workers.

A. fischeri, like A. fumigatus, is thermotolerant but is unable to grow at or above 50°C, and therefore can be considered to be weakly thermotolerant.

Aspergillus spp. 2, 3, 4 and 5.

These species can all be included within the Aspergillus fumigatus group and due to the extremes of variation encountered in this species, Aspergillus spp. 2, 3, 4 and 5 can all be designated as mutant strains of A. fumigatus.

Aspergillus sp. 2

Isolated from a coal spoil tip and from the air spora. This species is stable in culture and varies from A. fumigatus in a number of respects.

Colony growth on potato dextrose agar and Czapek-Dox

agar is rapid at 40°C and colony colour varies from white to pale buff (see Plate 14a), with a cream reverse. Colonies are extremely floccose and heavy sporing, and the petri plate lid may be covered in a white spore film. Conidiophores are produced from the aerial mycelium and from the substrate mycelium, they are smooth, hyaline, 100-350 μ in length and 2-4 μ wide. The shape of the vesicles is reminiscent of A. fumigatus although slightly more spherical, mostly 10-16 μ across and vesicles are fertile on the upper half only, (see Figure 35). Sterigmata are in one series, very short, 3-5 μ by 1.5-2.5 μ. Conidia are hyaline, white in mass, smooth, globose and very small, 1.5-2.0 μ, but occasionally larger spores are found (see Figure 35c). Spore columns are never formed.

Due to the large number of differences between this strain and normal strains of A. fumigatus it was originally considered that this was a separate Aspergillus species. Buff-coloured mutants of A. fumigatus and the floccose nature of certain isolates is not unknown but the production of small, squat sterigmata and small, smooth conidia by the present strains suggest significant variation. However, upon further

a. Aspergillus sp. 2

Plate 14 7 days on Czapek-Dox agar at 40°C.

b. Aspergillus sp. 3

Figure 35

Aspergillus sp. 2

a. Young vesicle.

b. Conidiophore heads showing small phialides.

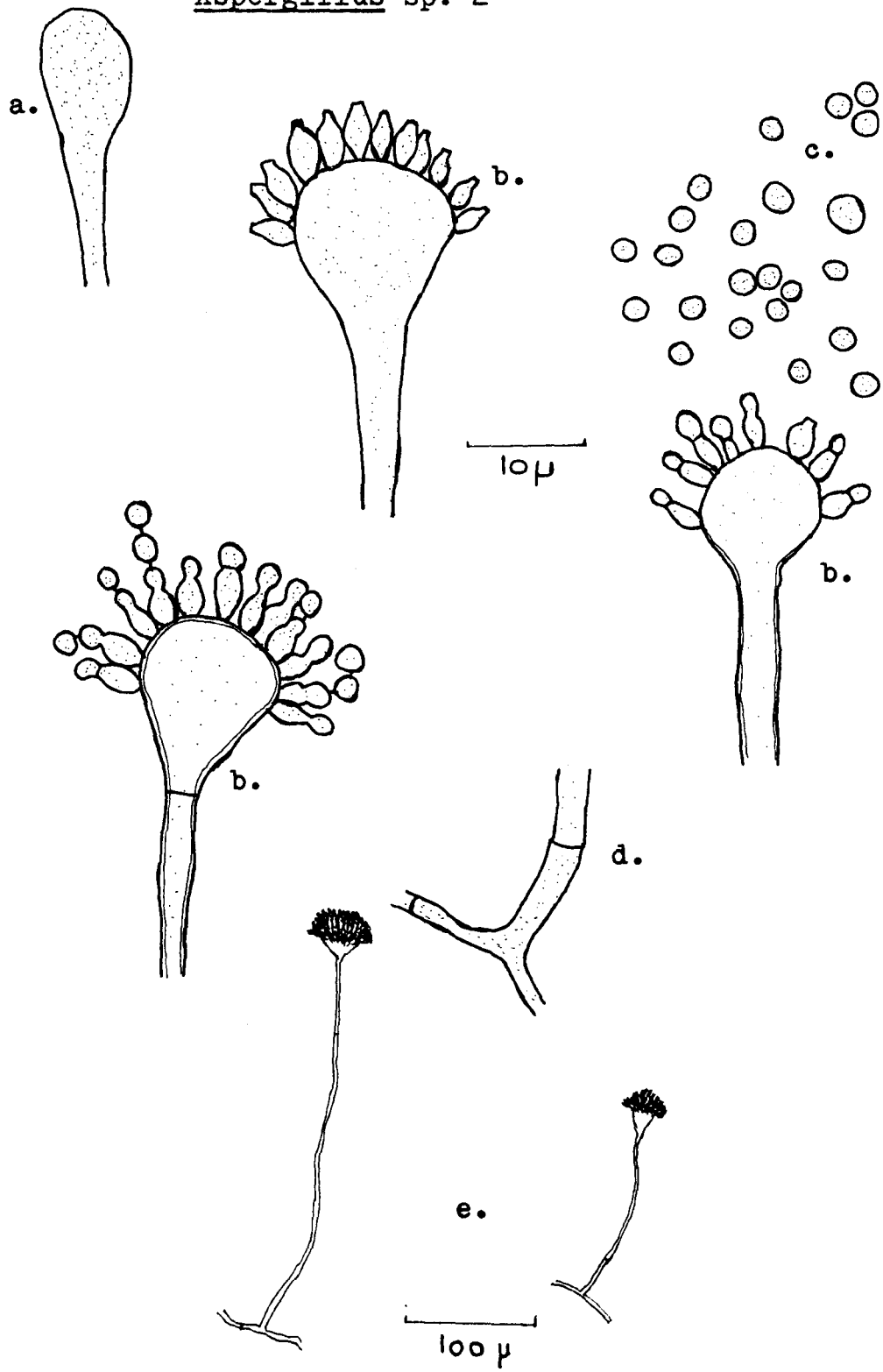
c. Conidia; small, smooth.

d. Foot cell.

e. Habitat view.

Figure 35

Aspergillus sp. 2



discussion with Dr. A.C. Stolk and Dr. G.A. de Vries, Centraalbureau voor Schimmelcultures, it was finally decided to classify them as mutant strains of A. fumigatus. Dr. de Vries has shown me drawings of a large number of strains or varieties of A. fumigatus showing the tremendous amount of variation not only in colony appearance but also in conidial shape and size, encountered in isolates of this species. He is at present investigating the anomalies of A. fumigatus with a view to publishing these findings. It is thought wise therefore, to include Aspergillus sp. 2 as a mutant strain or a variety of A. fumigatus. From a comparison of the temperature-growth relationships, Aspergillus sp. 2 would not appear to be as thermotolerant as normal strains of A. fumigatus.

Aspergillus spp. 3 and 4

Isolated from a coal spoil tip and from the air spora.

Strains of Aspergillus spp. 3 and 4 develop well on potato dextrose agar, Czapek-Dox agar and malt agar at

40°C. Mycelium is well developed and colonies are initially white or grey with the mycelium aggregated into ropes or strands from which the conidiophores arise. Conidiophores also arise directly from the substrate. In older cultures of Aspergillus sp. 3 green shades develop and coremial-like structures have been observed (see Plate 14b). The asexual stage of this species is typical of A. fumigatus and the heads are densely crowded, although mis-shaped heads bearing few sterigmata also occur (see Figure 36a). However, the colonies of Aspergillus sp. 4 remain greyish-white (see Plate 15a) due to light sporing, and the surface mycelial growth is occasionally yeast-like. The conidiophores usually arise from the aerial mycelium and tend to be short, 20-80 μ (see Figure 37a). The vesicles are normally very small, 4-7 μ and bear few sterigmata. These range from 5-7 μ long by 1.5-2.5 μ wide. The conidia are smooth and spherical but similar in size to those of A. fumigatus. Spore columns are never formed and the spores are produced in short chains.

It is considered that Aspergillus spp. 3 and 4 are mutant strains or varieties of A. fumigatus which have a funiculose-hyphal arrangement. The temperature-growth

a. Aspergillus sp. 4

Plate 15 7 days on Czapek-Dox agar at 40°C.

b. Aspergillus sp. 5

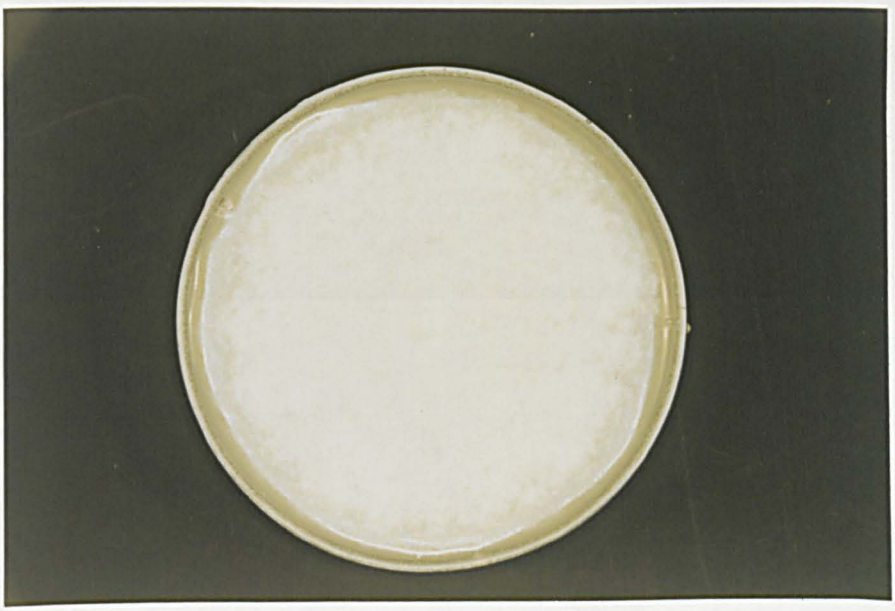
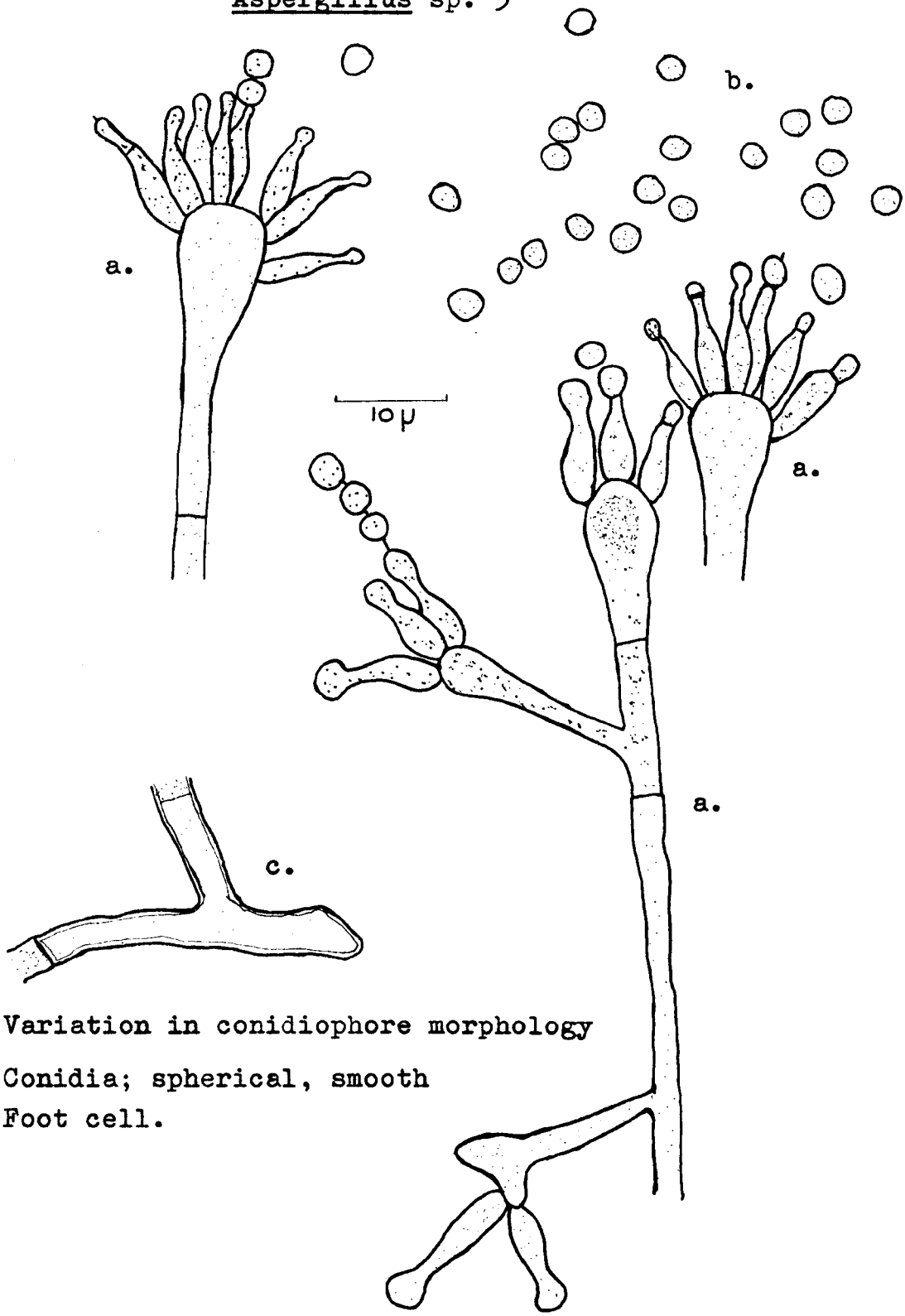


Figure 36

Aspergillus sp. 3



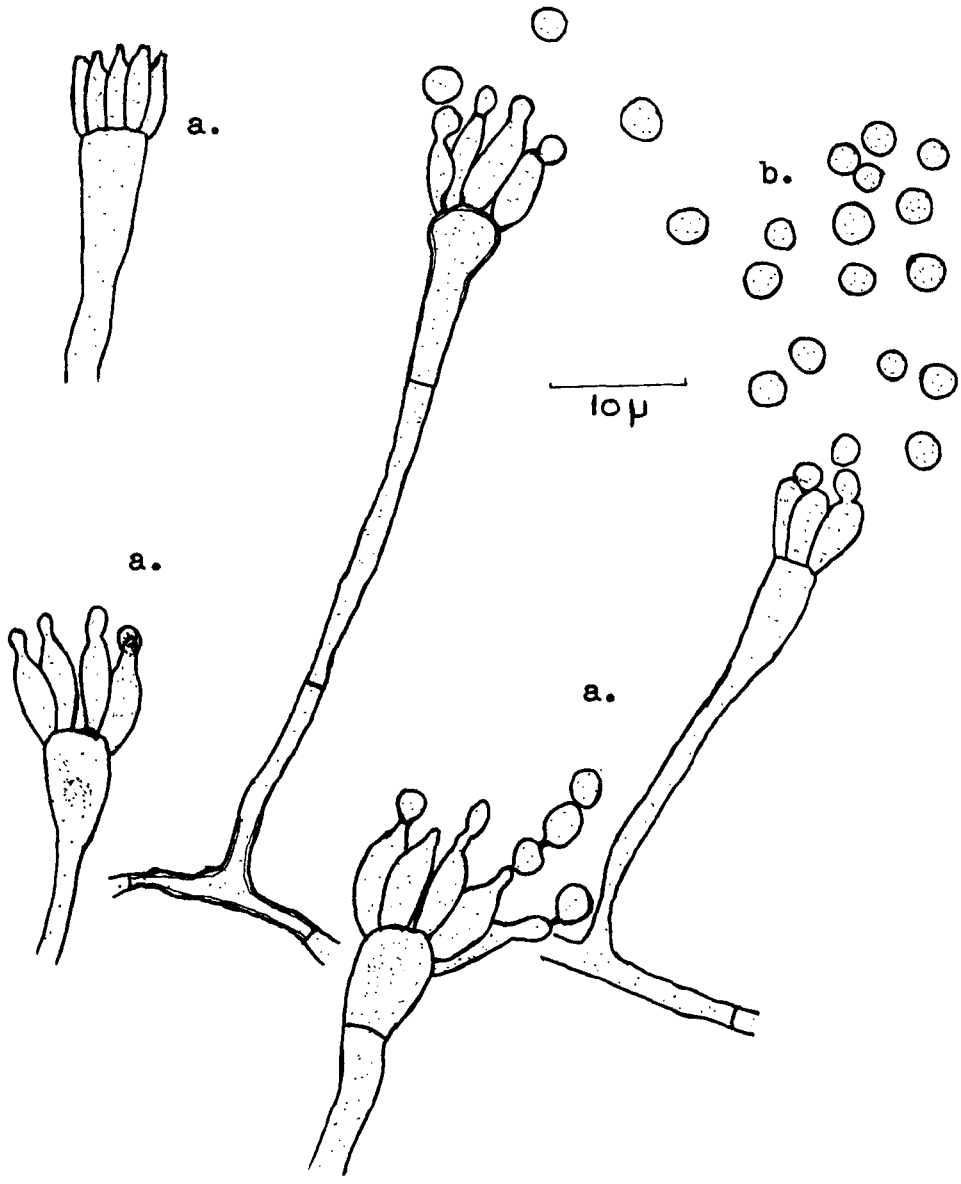
a. Variation in conidiophore morphology

b. Conidia; spherical, smooth

c. Foot cell.

Figure 37

Aspergillus sp. 4



a. Conidiophores with small vesicles bearing a small number of phialides.

b. Conidia; smooth.

relationships of these strains also differ slightly from normal strains of A. fumigatus (see Chapter IV).

Aspergillus sp. 5

Isolated infrequently from a single coal spoil tip.

Growth at 40°C on potato dextrose agar, Czapek-Dox agar and malt agar is extremely rapid and low, white colonies are formed (see Plate 15b), cream reverse. White hyphal aggregations or knots are formed but cleistothecia or initials have never been observed. Colonies are very light-sporing and short conidiophores are produced in clusters from the mycelial aggregations. Occasionally a pale green colouration is evident, particularly on malt agar. Spores are produced sparingly in short chains but never in columns. However, at low temperatures (20°C) colony colour and appearance is radically different. Growth is slow and a white aerial mycelium is produced, but this is soon overgrown by the development of numerous columnar conidal heads. From Figure 38 it can be seen that the shape of the vesicle and the sterigmatal arrangement is very similar to that of A. fumigatus. The conidia are primarily smooth but occasional echinulate spores are observed, in size and

Figure 38

Aspergillus sp. 5

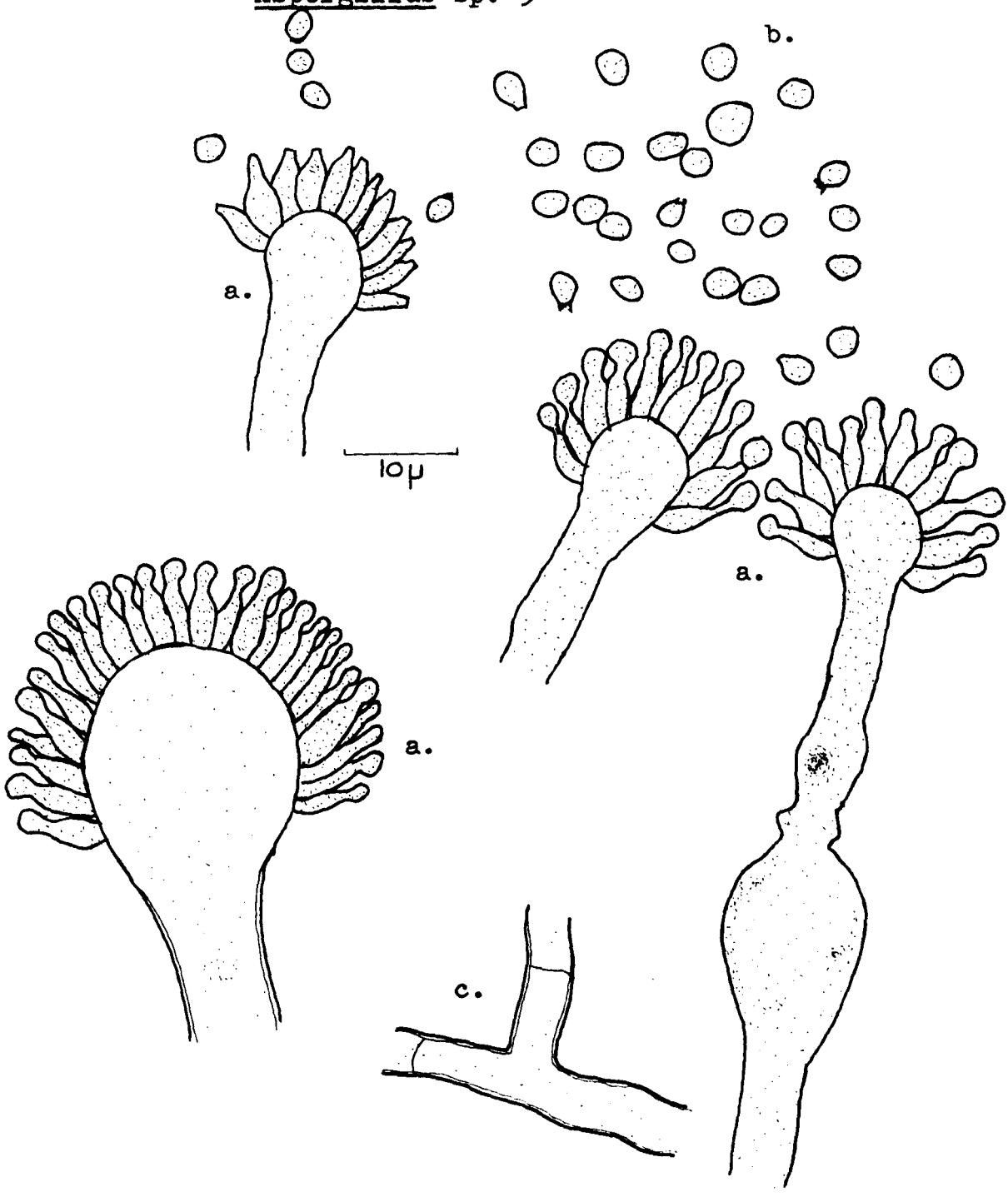
a. Conidial heads typical of A. fumigatus.

b. Conidia, showing variation in spore size and shape.

c. Foot cell.

Figure 38

Aspergillus sp. 5



shape the spores are variable.

It was originally thought that this species was a strain of A. fischeri but cleistothecia have never been observed and the dense conidial production at lower temperatures is not typical of this species. It is, therefore, thought that this represents a mutant strain of A. fumigatus which responds in its colony appearance and morphology to large changes in temperature. This is particularly noticeable when white, light sporing colonies grown at 40°C are left at room temperature for a number of days, after which time the dark green, columnar conidial heads typical of A. fumigatus predominate, altering the entire colony appearance. The temperature-growth relationships of this strain, as shown in Chapter IV, are similar to those of normal strains of A. fumigatus.

Calcarisporium sp. (unidentified Hyphomycete)

Isolated infrequently from a single coal spoil tip (Shelton), exclusively from the warm areas.

At 40°C on potato dextrose agar, growth is relatively rapid compared with that at 25°C. White cotton-like floccose colonies are produced (see Plate 16a) and

older colonies may develop pale buff shades and in very old cultures a vinaceous diffusate is sometimes present in the medium. This species has a similar growth pattern on a variety of media, although on yeast-starch agar the colony outline tends to be irregular and feather-like. On malt agar colony growth is very dense with a cream reverse.

The aerial mycelium is thin, (1.5)2-3 μ in diameter hyaline, septate, smooth and the sporogenous cells arise directly from this mycelium. The sporogenous cells may be borne in pairs or may also occur singly (see Figure 39), and are swollen at the base, 1.5-3.5 μ, tapering to an almost needle-like thread less than 1 μ in diameter. They are variable in length, 20-70 μ, with a slightly inflated apex from which the conidia are developed usually in acropetal succession. The conidia are borne on conspicuous denticles which usually remain after the conidia have been dislodged (see Figure 39a), denticles may be regularly or irregularly arranged. Conidia are hyaline, smooth, oval to sub-globose, 6-9 μ by 4-6 μ, with a distinct apiculus. Chlamydospores are occasionally found along the aerial or substrate mycelium and these are of irregular shape, thick-walled,

Figure 39

Calcarisporium sp.

a. Needle-like sporogenous cells arising singly or in pairs from the aerial mycelium.

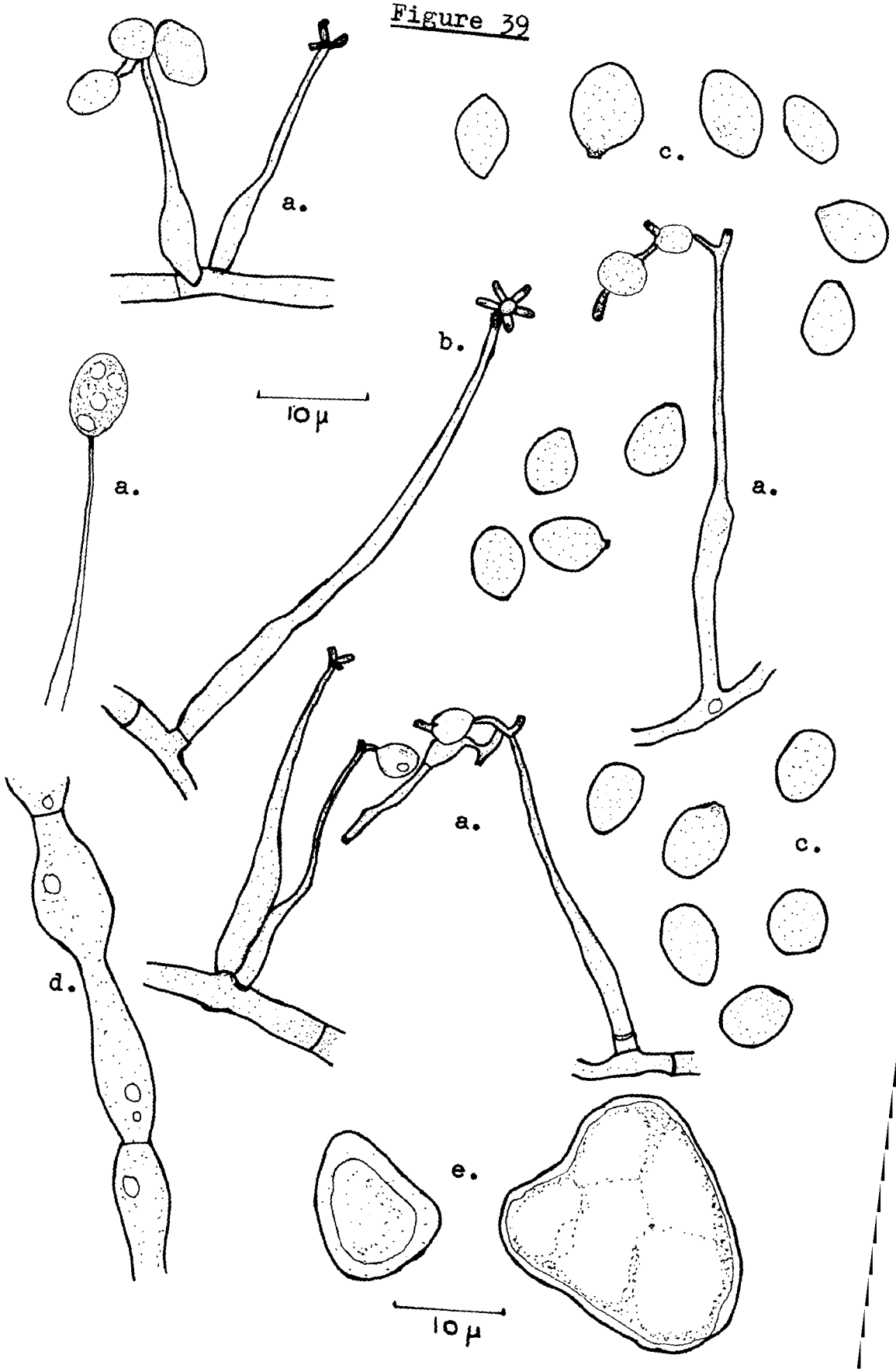
b. Denticle formation at tip of sporogenous cell.

c. Conidia.

d. Swollen mycelium from which chlamydospores arise.

e. Chlamydospores.

Figure 39



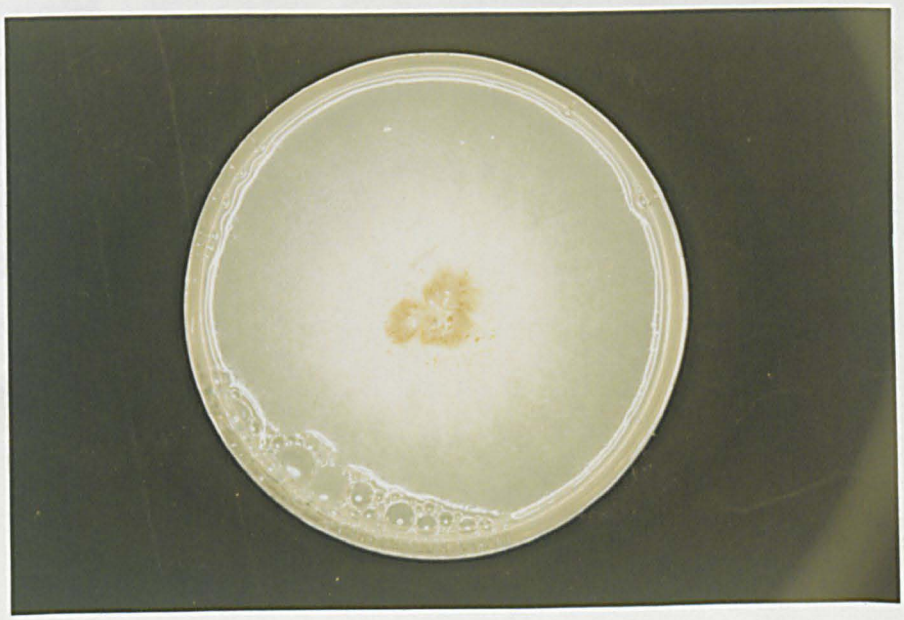
often with dense contents and mostly 10-30 μ in diameter (see Figure 39e).

This species was identified by Dr. G. Hennebert (Centraalbureau voor Schimmelcultures and University of Louvain) who placed it in the genus Calcarisporium Preuss. He suggested that this represented a new species and the representative species of the genus Calcarisporium have subsequently been examined, viz: C. arbuscula Preuss CBS 144.52, C. pallidum Tubaki CBS 130.58. The type species, C. arbuscula, is characterised by the sporogenous cells being arranged in whorls on upright conidiophores, as described by Hughes (1951). A careful examination of strains of the present Calcarisporium sp. has not revealed the presence of distinct, upright conidiophores and true whorls are not generally produced. The sporogenous cells usually occur in pairs (reduced whorls) or singly. It is hoped to publish shortly a valid description of this species under the name Calcarisporium thermophile, due to its ability to grow at elevated temperatures (see Chapter IV). Calcarisporium species are usually associated with the decaying fructifications of higher fungi and have rarely been isolated from soil. Cultures of this species have

a. Calcarisporium sp.
7 days on potato dextrose agar at 40°C.

Plate 16

b. Cephalosporium sp. 1
10 days on potato dextrose agar at 30°C.



been deposited at the Centraalbureau voor Schimmelcultures.

Cephalosporium sp. 1

Isolated infrequently from chalk grassland and more frequently from the air spora.

Growth is rapid at 40°C on a wide variety of media and dense, white, floccose colonies are produced. The colony margin is regular but sectoring may occur and patches of low, thin hyaline mycelium are interspersed amongst the more vigorous areas. Colonies have a cream reverse but may develop shades of brown with the development of light brown to dark brown sclerotial bodies. These usually develop only at the lower temperatures (20-30°C) and occur in groups initially at the centre of the colony (see Plate 16b) but later may spread over the entire agar surface.

The aerial mycelium is thin, 2-5 μ in diameter, hyaline, septate and a Cephalosporium-like asexual stage is borne directly from this mycelium and distinct conidiophores are not usually observed. The sporogenous branches are variable in length, 5-40 μ and are usually swollen towards the apex. From these swellings one to five finger-like projections occur, usually enclosed in

a slimy spore-ball, 10-20 μ in diameter (see Figure 40a). The projections measure 7-8 μ by 1-2(2.5) μ, and may also occasionally occur singly along the mycelial branches. Spores are cylindrical to oblong, hyaline, smooth, mostly 3-5 μ by 1.5-2 μ.

Large, swollen, irregularly-shaped chlamydospores occur in abundance on the agar surface. These have dense contents and measure 10-50 μ in length. Similar structures may also be found inside the sclerotia. Sclerotia are light brown to dark brown, globose to flask-shaped, with few or no appendages but with an uneven appearance to the surface (see Figure 40d). They range from 100-300 μ in diameter but mostly average 150-200 μ. Occasionally, however, larger sclerotia of variable shape occur and in some cultures areas of continuous brown, irregular cells, identical with those comprising the sclerotial wall, form on the agar surface. It is thought that this may represent a pseudoparenchymatous tissue formation. The sclerotia were initially considered to be ascocarps but asci or ascospores have never been observed and they should, therefore, be classified as vegetative structures.

The finger-like projections appear to fragment,

Figure 40

Cephalosporium sp. 1

a. Spore-balls, showing finger-like projections.

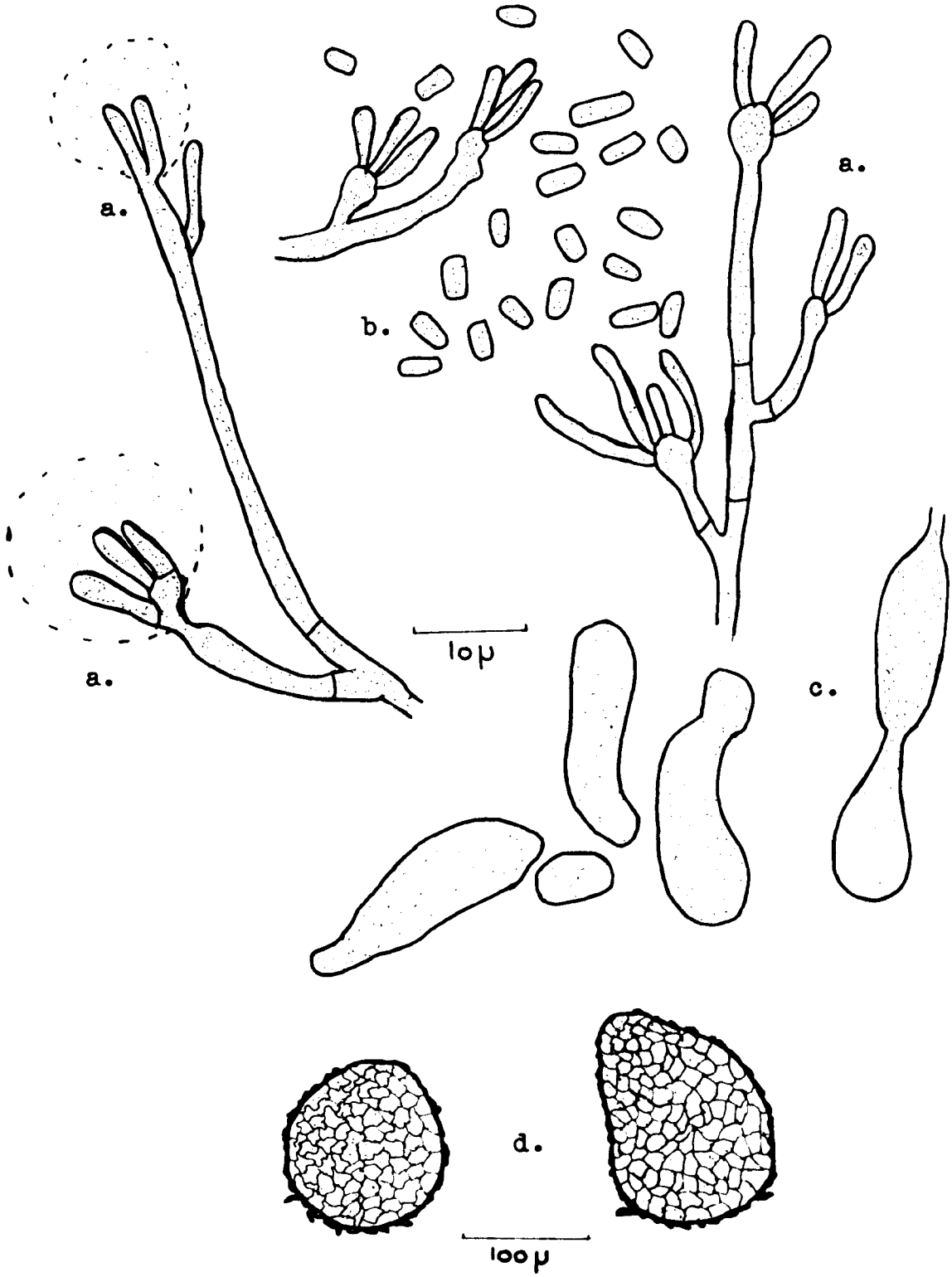
b. Arthrospores.

c. Swollen cells or chlamydospores occurring on the agar surface and in the sclerotia.

d. Sclerotia.

Figure 40

Cephalosporium sp. 1



giving rise to gloeoid spore-balls. The spores can, therefore, be more accurately termed arthrospores (or oidia) as distinct from phialospores as produced by members of the Cephalosporium group. Dr. W. Gams has confirmed that this does not represent a Cephalosporium species and because of the pattern of septation (see Figure 40a) he regards this species as belonging to the Basidiomycetes. An attempt has been made to trace this species using the key proposed by Nobles (1965) but with little success. Arthrospores are produced by a number of Basidiomycetes but their mode of formation appears to be different from that of the present species. Certain Polyporus species produce similar oidial stages but this interesting species cannot be accurately classified until details of the fruit-body are forthcoming. It is hoped to try and culture this species on a wide variety of natural substrates in order to obtain the sexual stage. Apinis (1965) isolated a thermophilous Coprinus species from coastal grasslands but it would appear to be less strongly thermotolerant than isolates of the present species (see Chapter IV for the temperature-growth relationships of Cephalosporium sp. 1). This species, C. delicatulus, has an oidial stage and a

sclerotial stage generally similar to those of Cephalosporium sp. 1 and it may be that the latter represents a reduced Coprinus species.

Cephalosporium sp. 2

This species was isolated from a single coal spoil tip and it is represented by only two isolates.

At 40°C on potato dextrose agar, growth is rapid and white, bushy colonies are initially produced. However, colonies rapidly turn pink to dark red due to the production of numerous exudate droplets, which also stain the agar medium. This is very conspicuous on oat-meal agar. Older colonies become light-brown or dark-brown. At higher temperatures colonies are low, dense, white at first becoming dark green or brown with age, especially towards the centre. The red pigmentation is very much reduced or lacking at these temperatures.

Cephalosporium-type structures are formed directly on the bushy aerial mycelium and conidiophores are not distinct. The sporing mycelium may appear slightly uneven or roughened and often swollen hyphal cells develop from which the phialides arise as short branches, 3-10(15) μ long by 1.5-2 μ, producing spores from a

slightly tapering apex. Phialides vary in form from short, swollen or inflated structures to long, cylindrical structures (see Figure 41). Spores usually aggregate into slimy spore balls, 4-6 μ in diameter, and are mostly oval, occasionally globose, 2.5-3.5 μ by 1.5-2 μ, smooth, hyaline and some spores have a definite apiculate region. The aerial mycelium is hyaline, 2-3 μ in diameter, occasionally up to 5 μ in the swollen condition. Brown hyphae develop in older cultures, 5-6 μ in diameter. Globose to spherical, brown crystalline deposits, 10-30 μ in diameter, occur along parts of the mycelium in a bead-like arrangement. Crystals of various shapes and sizes occur abundantly in the medium and may also be deposited directly on the surface of the mycelium (see Figure 41d). Dark brown almost black, flat, sclerotial bodies are found in groups tightly adhering to the agar surface. These are composed of swollen, brown hyphae (see Figure 41c) and they range from 100-400 μ in length.

This species does not show the characteristic long, tapering phialides of the Cephalosporium series. It would appear that this species shows close affinities with those of the genus Phialophora Medlar, viz: mostly short, slightly swollen phialides, with ovoid

Figure 41

Cephalosporium sp. 2
(*Phialophora*)

a. Phialide formation.

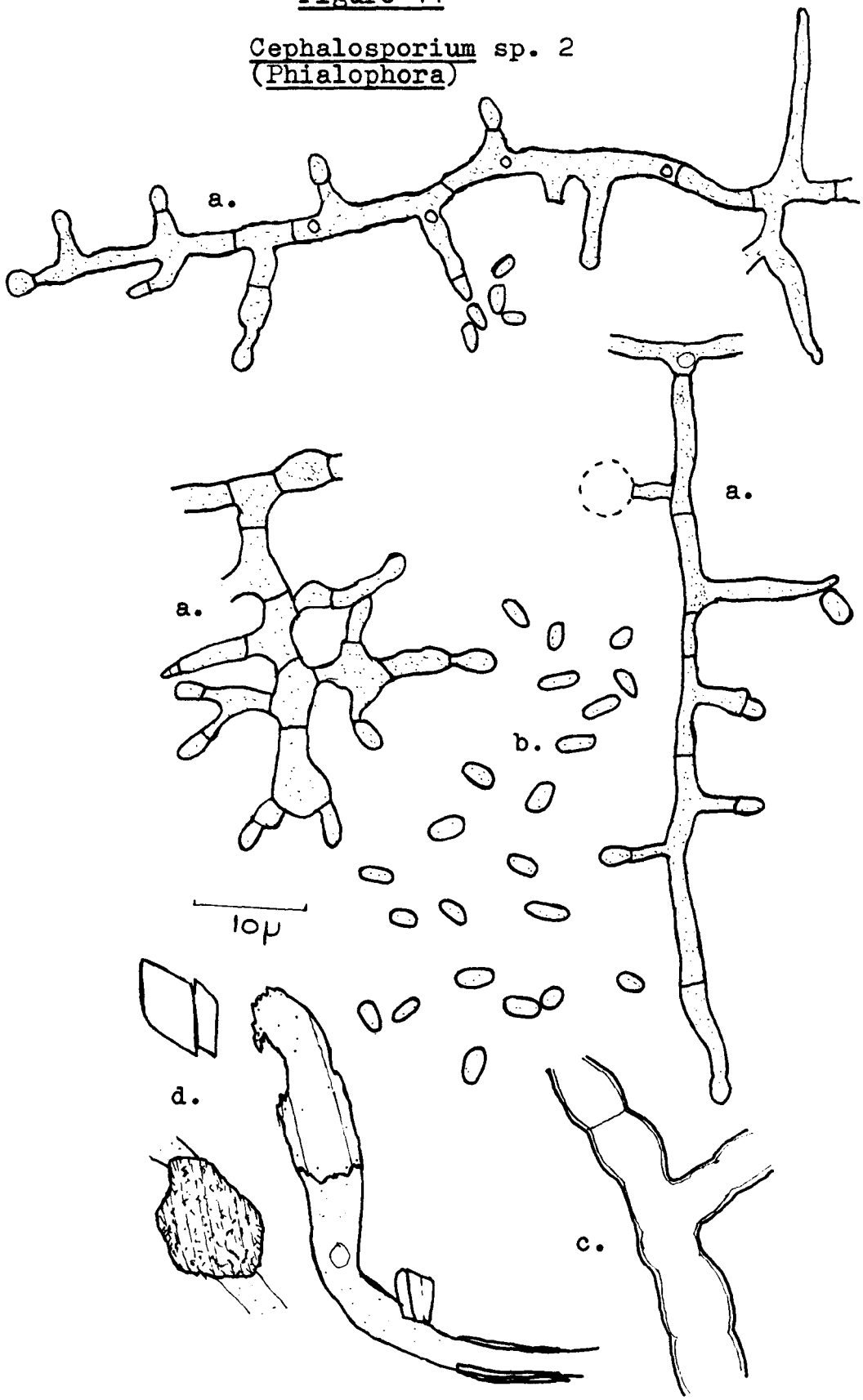
b. Conidia.

c. Swollen hyphal fragment, comprising the sclerotial bodies.

d. Crystal structures in the medium and on the mycelium.

Figure 41

Cephalosporium sp. 2
(Phialophora)



spores gathering in large gloeoid balls. The diagnostic feature of this genus is the presence of a distinct apical collarette on the phialides. This collarette, however, has never been observed in the sporing structures of the present species. Nevertheless, Barron (1968, p.256) notes that the apical collarette is lacking in some species and the phialospores at the apex of somewhat tapering phialides. Barron also reports that many Ascomycetes are known which possess Phialophora-like conidial states. Sexual stages have never been observed in the present species, but it may be that this represents the asexual stage of an Ascomycete species. It is difficult, therefore, to classify this species further although it does not conform with any of the Phialophora species so far described. Cultures have been deposited at the Centraalbureau voor Schimmelcultures.

Chrysosporium pruinosum (Gilman and Abbott) Carmichael Can. J. Bot. 40, 1167-1168, 1962.

This species was infrequently isolated from the air spora. A detailed morphological and temperature study was not made due to the infrequent occurrence and

the low maximum temperature for growth of this species. The actual temperature-growth range of C. pruinosum is approximately 10-45°C, i.e. weakly thermotolerant. A type culture of Sporotrichum pruinosum Gilman and Abbott, IMI 74,692 was examined and taxonomically was found to compare favourably with the present isolates. At 30°C on potato dextrose agar and yeast-starch agar, low, thin, white to cream, slightly floccose colonies develop. The conidial stage is usually poorly represented but chains of spherical chlamydospores occur abundantly.

Chrysosporium sp.

Strains of this species were originally designated Chrysosporium spp. 1 and 2, on the basis that several strains isolated exclusively from the air spora appeared to have slightly different cultural features, including the presence of dark, sterile bodies, and also different temperature-growth relationships (see Chapter IV). However, after a careful examination of the conidial stages of all the strains involved it was concluded that they represented a single species and these will be described under the name of Chrysosporium sp.

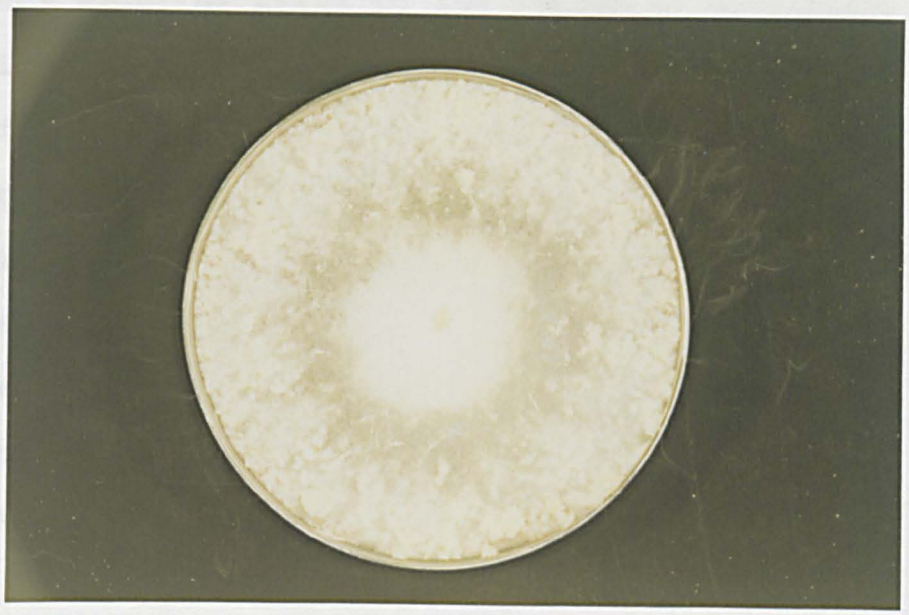
Growth at 40°C on potato dextrose agar is extremely rapid and white, floccose colonies are produced after several days. These consist of tree-like aerial mycelial strands which become extremely dense as a result of heavy sporulation (see Plate 17a). Colonies are irregular, feather-like in appearance and a pink diffusate is usually apparent in the agar medium, the density of this strain depends on the individual strain. Older colonies become yellow or dirty yellow (see Plate 17b) and mealy as the aerial mycelium dies down, with a cream to light brown reverse. A cream, dense, substrate mycelium often becomes apparent and this may darken with age. Very old colonies appear light brown or even green in areas, with a dark brown reverse, and this is particularly characteristic of those strains which produce secondary structures.

The mycelium is hyaline, septate, smooth, 2-4 μ in diameter. Conidiophores are usually poorly differentiated although initially the tree-like, mycelial clumps produce conidia only towards the apex. However, in older cultures, conidia are produced practically from any part of the aerial mycelium. Conidia are produced laterally or terminally from the mycelium, either

a. 3 days on potato dextrose agar at 45°C.

Plate 17 Chrysosporium sp.

b. 7 days on potato dextrose agar at 45°C.



directly or on short processes which remain as conspicuous scars after the conidia have been shed (see Figure 42b). Conidia are hyaline to pale yellow, globose to cylindrical, often truncate, and smooth-walled. Globose conidia measure 5-8(9) μ by 3-4.5 μ; cylindrical conidia measure 9-15 μ by 3-5 μ. However, as can be seen from Figure 42, conidial shape and size is extremely variable. Chlamydospores are profusely formed in older cultures and take the form of spherical or globose swellings, mostly 8-20 μ in diameter but larger, more irregular cells occasionally occur along the substrate mycelium.

Certain strains produce ascocarp-like structures irregularly and these are usually found amidst the low, compact mycelial areas in the centre of the colony. Initials are abundantly produced (see Figure 42d) but few mature bodies occur. These are dark brown, spherical, thin-walled, more or less naked, although swollen cells may occur at the edge, 150-300 μ in diameter. Numerous cultures have been examined on a variety of media but asci or ascospores have never been observed. An indication that these may represent sclerotial structures is given when larger, more

Figure 42

Chrysosporium sp.

a. Method of spore formation.

b. Conidia; diverse shapes and sizes.

c. Chlamydospores, usually in chains.

d. 'Ascocarp' initial.

e. Brick-shaped cells typical of the mature ascocarp wall.

f. Swollen cells surrounding the ascocarp.

Figure 42

Chrysosporium sp.

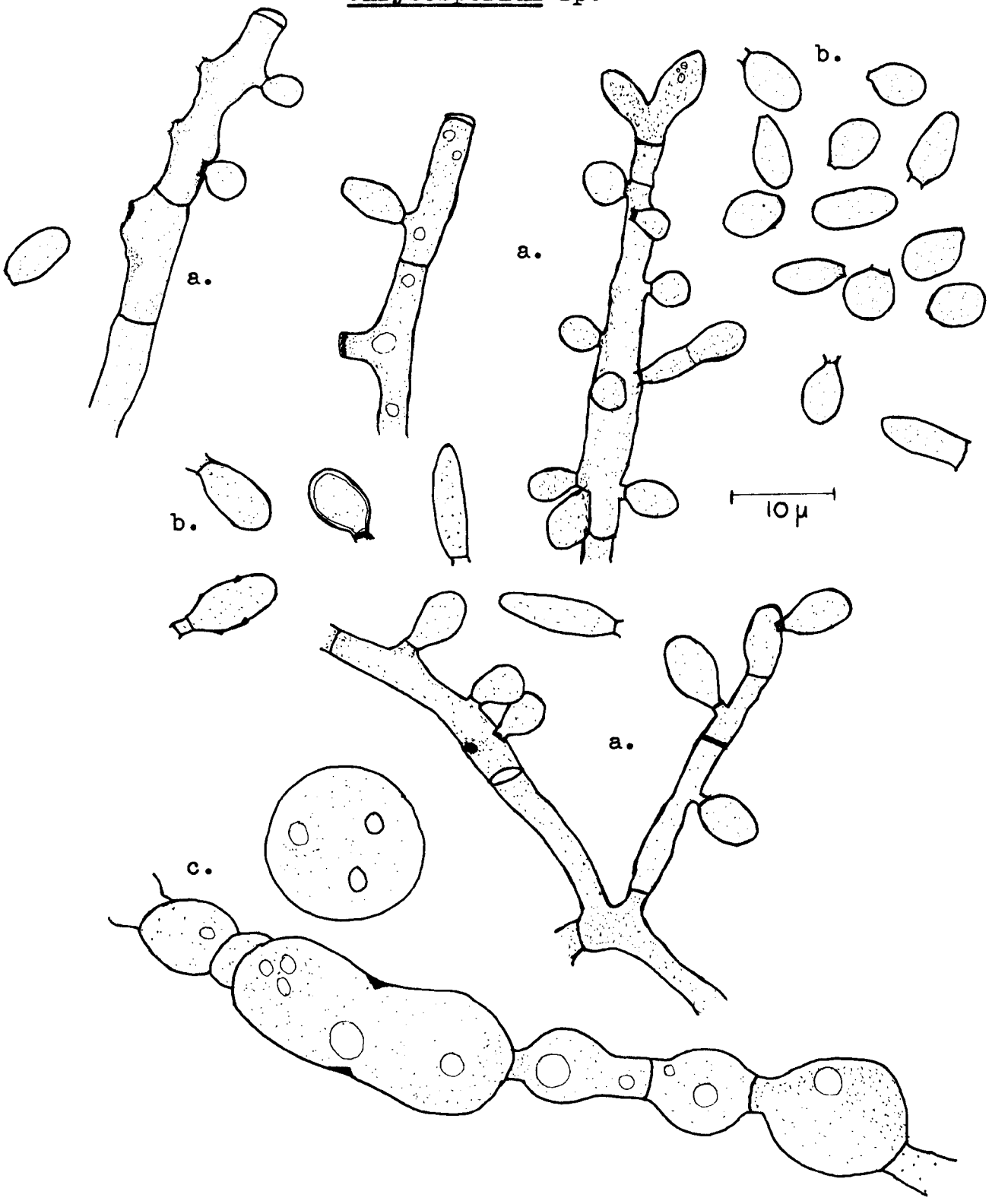
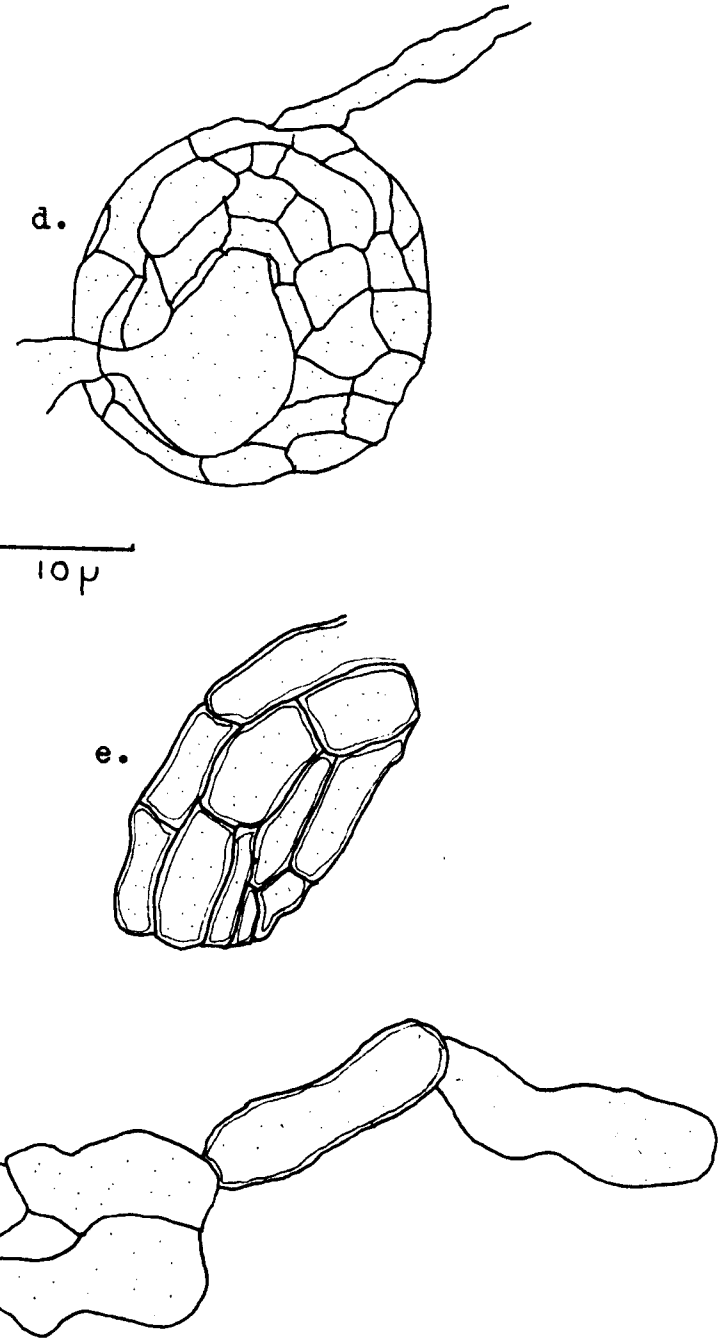


Figure 42

Chrysosporium sp.



irregular structures, up to 500 μ in diameter, are occasionally observed. However, the regular cells comprising the thin wall (see Figure 42e) of these structures and the general conformity in shape suggests that they may represent the sterile sexual stage of an Ascomycete.

Certain species of the Gymnoascaceae are known to produce Chrysosporium-like asexual stages and these occur in the genera Actinodendron, Ctenomyces and Shanorella. However, until the sexual stage is completely studied the present species must be placed in the genus Chrysosporium. A monograph of this genus has been presented by Carmichael (1962) but none of the species described in this monograph compare with the present species. A number of Chrysosporium species are known to grow at elevated temperatures but so far no species have been reported as being thermophilic. The temperature-growth relationships of the present strains of Chrysosporium sp. have been previously discussed (see Chapter IV) and notable differences were found to exist. Most strains were found to be strongly thermophilic (i.e. no growth below 25°C) and the remainder were classified as weak thermophiles, although all

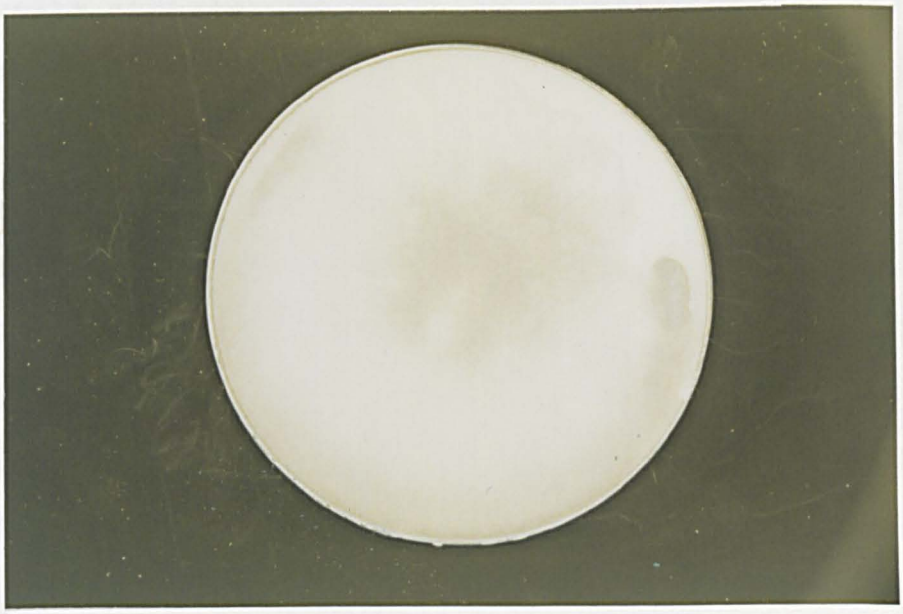
strains represented true thermophiles (i.e. no growth below 20°C).

Because of the thermophilic nature of this species and its very slow growth at normal isolation temperatures it may be that it represents a new Chrysosporium species. Numerous isolates have been obtained and it would appear to be a species with a wide distribution. This species has also been found to be strongly keratinophilic and it may represent an important part of the microflora of keratinous substrates, commonly found in nature. This species will be designated Chrysosporium thermophile due to its high temperature requirements for growth.

Geotrichum sp.

This species was isolated several times from a coal spoil tip profile.

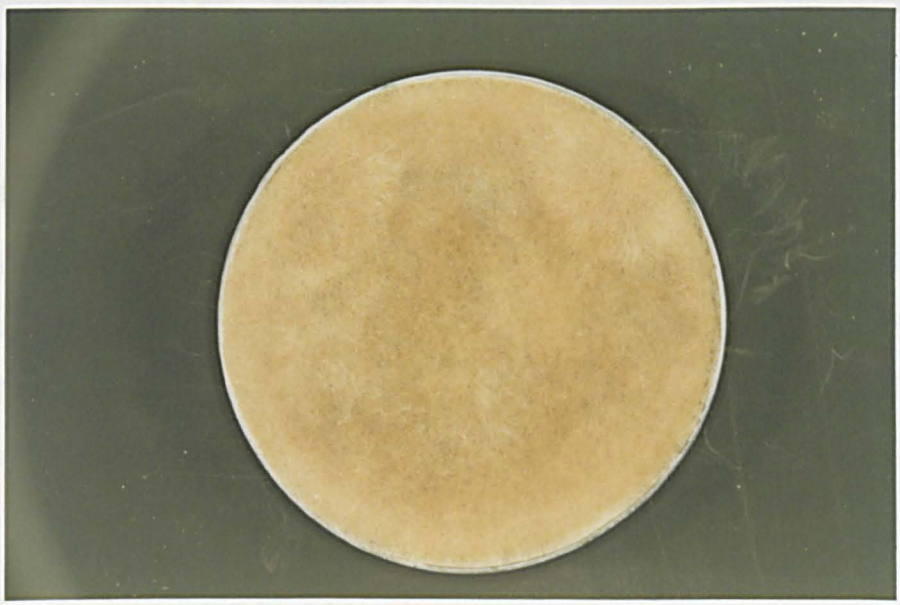
On potato dextrose agar and yeast-starch agar at 40°C growth is extremely rapid and dense, floccose, cotton-like, almost snow-white colonies are produced. With age, colony colour may become buff, cream on reverse. On the same media at 25°C growth is slower and the colonies are floccose, pale brown at first,



a. 7 days on potato dextrose agar at 40°C.

Plate 18 Geotrichum sp.

b. 14 days on potato dextrose agar at 25°C.



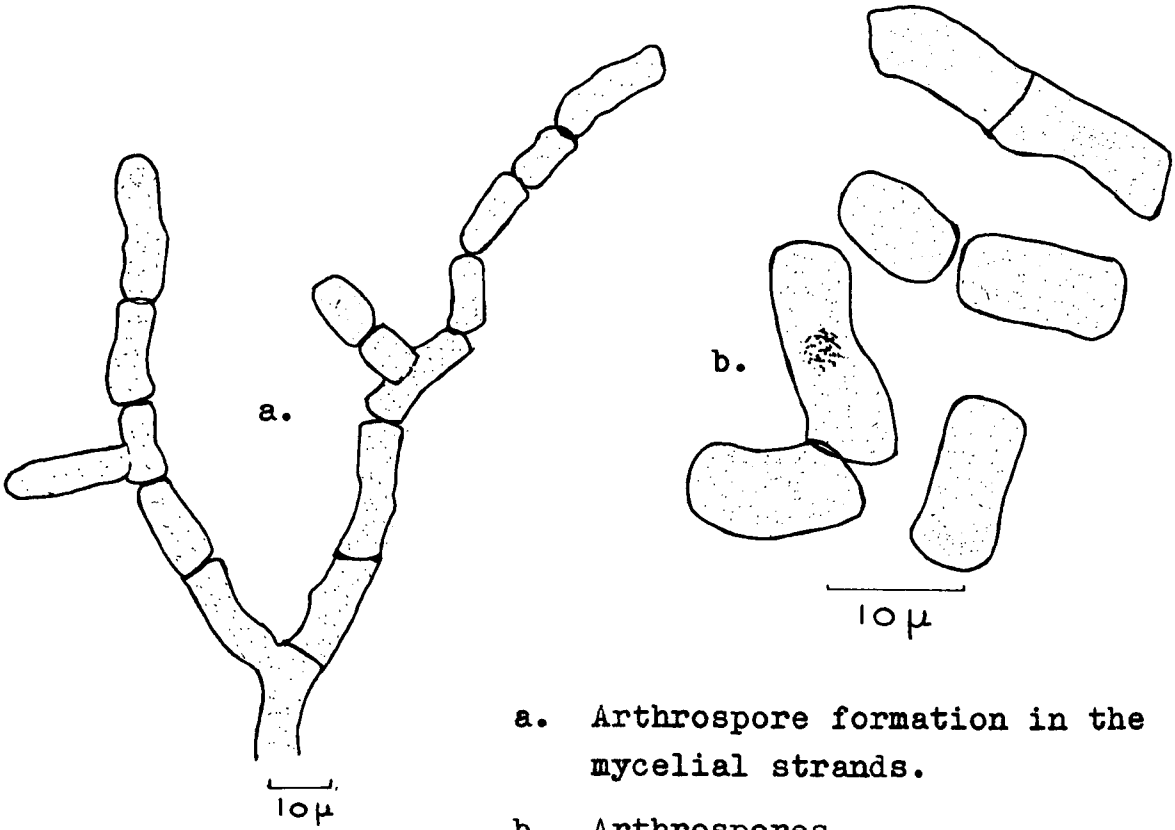
gradually changing to brown or ginger-brown. In older colonies the mycelium is stiff and wire-like, ginger coloured with a dark brown reverse (see Plate 18a and b).

Sporing is very variable and occurs from 20-40°C, although lower temperatures (20-30°C) induce most frequent sporing. Tree-like hyphal strands develop primarily at the edges of the colony (i.e. against the sides of the petri plate) and spores are usually formed in dense, white clumps, having an almost fluffy appearance, and are found usually against the lid of the petri plate. The spores are formed at the tips of the aerial mycelium (see Figure 43a) and are very variable in size and shape; mainly cylindrical with truncate, slightly convex walls, and measure mostly 10-20 μ by 4-7 μ, although smaller, almost globose spores are occasionally observed.

The ginger-coloured hyphae develop primarily at the lower temperatures and have a characteristic morphology. Hyphae are septate, 4-10 μ in diameter, although hyphae up to 15 μ are not uncommon, and become thickened or roughened due to the decomposition of spherical encrustations over the entire hyphal surface (see Figure 43c).

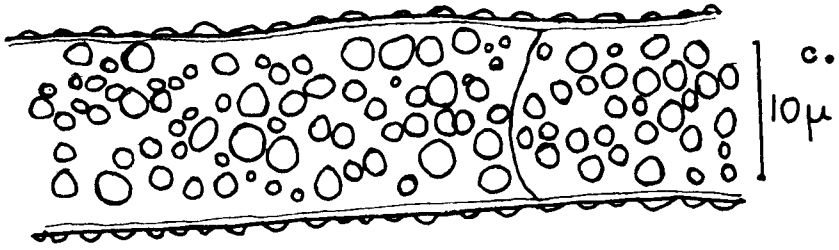
Figure 43

Geotrichum sp.



a. Arthrospore formation in the mycelial strands.

b. Arthrospores.



c. Hyphal strand from old culture; golden to ginger-brown with conspicuous depositions along the wall.

A discussion of this species with Dr. G. Hennebert and a subsequent comparison of cultures and morphological details with his strains of Geotrichum proved without doubt that this Geotrichum sp. is identical with Geotrichum arbustum (in editum). This name has recently been proposed by Dr. Hennebert and a description of this species will be published shortly. He has not grown his strains above 25°C but it would seem likely that they would also show thermotolerant properties.

Humicola grisea Traaen var. thermoidea Cooney and Emerson in Thermophilic Fungi, pp.74-79, 1964.

This species was infrequently isolated from a single coal spoil tip. On yeast-starch agar the jet-black colour of the colonies is very characteristic of H. grisea var. thermoidea. The conspicuous apiculus on the aleuriospores is another good taxonomic feature which serves to delimit this species from H. insolens. Morphologically these strains are similar to those described by Cooney and Emerson, although chains of chlamydospores of variable shape are also produced and to a certain extent this species was confused with

H. insolens. This has also recently been reported by Fassatiová (1967, p.81) who emended the original description of H. grisea var. thermoidea to include the frequent occurrence of chains of intercalary, globose, oval or elongated chlamydospores.

Humicola insolens Cooney and Emerson
in Thermophilic Fungi, pp.73-79, 1964.

This proved to be a relatively ubiquitous species and was especially abundant in manures and composts.

Growth on yeast-starch agar at 40°C results in a dense spore production and low, grey to almost black colonies are produced. However, the jet-black colour typical of H. grisea var. thermoidea is never developed. On potato dextrose agar at this temperature growth is low and almost entirely subterranean producing dark grey, light-sporing colonies. Globose and flask-shaped aleuriospores are present together with chains of intercalary spindle-shaped chlamydospores. Sizes agree with those given by Cooney and Emerson.

Fassatiová (1967) has examined species and strains of the genus Humicola and proposed certain emendations. A comparison of strains of H. fuscoatra and H. insolens was made and differences justifying their delimitation

as separate species were not forthcoming. However, the presence of flask-shaped aleuriospores and the characteristic spindle-shaped chlamydospores of H. insolens are considered to justify separation on a varietal basis, the new variety being designated H. fuscoatra var. longispora Fass. Non-thermophilic strains similar in morphology to H. insolens were examined and a further combination was considered necessary, viz: H. fuscoatra var. longispora forma insolens (Cooney and Emerson) Fassatiová, basionym; H. insolens Cooney and Emerson.

Torula thermophila Cooney and Emerson
in Thermophilic Fungi, pp.88-92, 1964.

This species is discussed in the section Humicola due to the doubt existing in assigning this species to the genus Torula. This problem is summarised by Fassatiová (1967, p.89) who examined strains of T. thermophila and subsequently considers them to be a form of H. grisea var. thermoidea which produces only globose chlamydospores. During the present study T. thermophila was isolated from a variety of habitats, including manures and birds' nests.

On potato dextrose agar at 40°C growth is rapid

and takes the form of chains of dark brown arthrospores or chlamydospores ramyfying through and on the surface of the agar. Areas of dense mycelium and spore formation occur, giving the colony a zoned appearance, a constant cultural feature on this medium. On yeast-starch agar jet-black colonies are formed.

The arthrospores are spherical, dark brown with thick walls and are produced in single or branched chains. Cooney and Emerson gave measurements of 8-12.5 μ (occasionally 17 μ) for spore diameter. However, the spores of the present isolates are consistently larger, the average diameter being about 14 μ and the range 9-18 μ. Cultures of this species have been deposited at the Commonwealth Mycological Institute, IMI 125,821.

From a comparison of the type species of Torula, T. herbarum Persoon, it is considered that the latter species should not be placed in this genus which is characterised by the production of chains of porospores which break up into phragmospores. A different method of spore formation is evident in T. thermophila which is typified by intercalary chlamydospores produced in single or branched chains; they may also occasionally develop as terminal swellings on the tips of short

lateral branches. The spores are liberated singly and never as phragmospores, i.e. a spore having two or more transverse septa. It would appear, therefore, that T. thermophila should be placed in the genus Humicola but its exact position is uncertain and it may be advisable to include it as an aberrant form of H. grisea var. thermoidea, although the chlamydospores are not entirely typical.

Humicola sp. 1

Isolated from a single manure heap undergoing extensive humification.

On potato dextrose agar at 40°C growth is fairly rapid, and characterised by an absence of aerial mycelium, growth being mainly subterranean. Colonies are initially grey but become dark grey and eventually black as spore production is increased. On yeast-starch agar growth is more rapid and the mycelium is well developed, colonies grey at first becoming yellow or green coloured and eventurally olive (see Plate 19a). On malt agar growth is slightly slower and grey-black, wrinkled colonies are formed, the spores may form a dark crust on the agar surface.

a. Humicola sp. 1
7 days on yeast-starch agar at 40°C.

Plate 19

b. Humicola sp. 2
7 days on malt agar at 40°C.

The vegetative mycelium is thin, septate, greyish, 1.5-4 μ in diameter. Swellings appear along the mycelium (see Figure 44a) giving rise to globose or spherical chlamydospores produced in chains (see Figure 44b). Mature spores are yellow or light brown appearing brown to dark brown in mass, measuring 10-15(18) μ in diameter with an average of about 14 μ. Also oval or spindle-shaped spores may be interspersed amongst the chains of globose spores, or are occasionally produced singly, these measure mostly 15-20 μ by approximately 10 μ. Side chains of chlamydospores are also produced and certain spores show slight apiculate areas.

This species should be classified in the genus Humicola as prescribed for Torula thermophila and indeed Humicola sp. 1 has certain affinities with the latter species. Chlamydospore development and morphology show excellent agreement; however, the spindle-shaped spores, a feature of Humicola sp. 1 are not so apparent in T. thermophila and the sizes differ somewhat. Nevertheless, it would seem likely that these species show similar characteristics but can be separated on several points, viz:

i. Colony appearance; relatively similar on potato

Figure 44

Humicola sp. 1

a. Stages in chlamydospore formation.

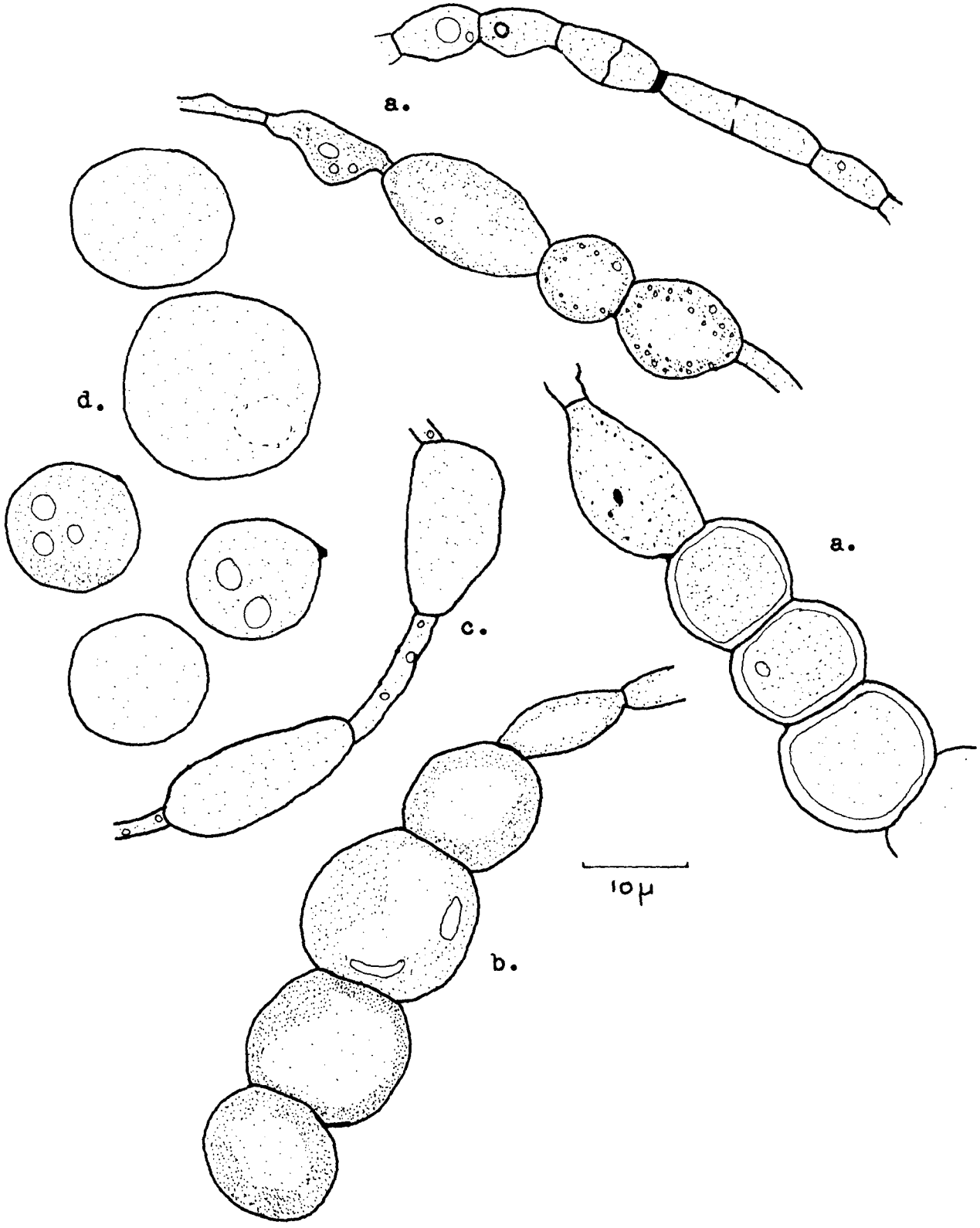
b. Chain of mature spores.

c. Elongated chlamydospores.

d. Spherical chlamydospores, showing variations in size.

Figure 44

Humicola sp. 1



dextrose agar but on yeast-starch they can readily be distinguished. The jet-black, soot-like colonies of T. thermophila contrasting with the grey to yellow-brown, slightly floccose colonies of Humicola sp. 1.

ii. Large spindle-shaped cells are a common feature of Humicola sp. 1, found to a lesser extent in T. thermophila where they are smaller.

iii. Temperature-growth relationships; T. thermophila is weakly thermophilic and cannot grow below 20°C, whereas Humicola sp. 1 is thermotolerant, having the ability to grow below 20°C, also the maximum temperature for growth is less than the former species.

It is possible, therefore, that Humicola sp. 1 is a reduced form of a true Humicola species, lacking the characteristic aleuriospore stage. However, it cannot be traced to any of the known species listed by Fassatiová (1967, pp.87-88).

Humicola sp. 2

Isolated from the same habitat as Humicola sp. 1.

On potato dextrose agar at 40°C growth is rapid and white, floccose colonies are produced, which gradually turn yellow or light brown. On yeast-starch agar the

growth rate is slightly increased and white to buff coloured colonies eventually turn to shades of yellow, depending on spore production. In very old cultures, thick, bright yellow to orange spore crusts develop on the agar surface. On malt agar the colonies remain white to buff in colour and floccose (see Plate 19b).

The mycelium is hyaline, septate, 1.5-3 μ in diameter. Golden yellow to pale brown aleuriospores are borne directly on the vegetative hyphae or more usually on short, lateral conidiophores which are septate and may be cylindrical or slightly inflated, (see Figure 45a). Mature aleuriospores are smooth-walled, generally globose, borne singly, ranging in size from 7-11 μ in diameter. The spores are always marked by a definite apiculate region. Intercalary, flask-shaped chlamydospores are formed and these fragment easily. They are yellow in colour and measure mostly 10-14(17) μ by 4-6 μ.

This species does not approximate any of the known species listed by Fassatiová and few species of the genus Humicola are known which produce light-coloured aleuriospores. The generally light-coloured colonies

Figure 45

Humicola sp. 2

a. Aleuriospore formation, direct from the mycelium or on short stalks.

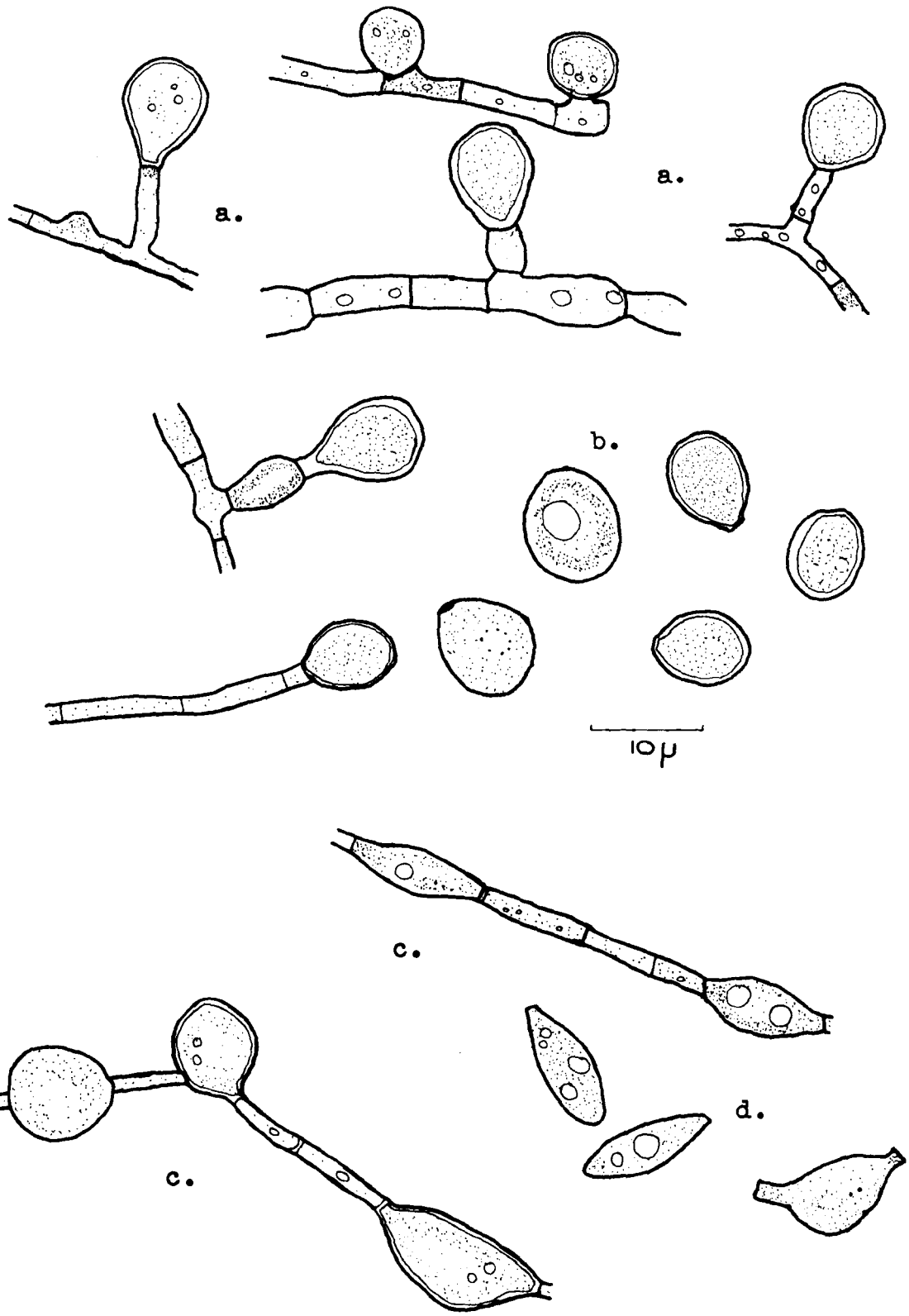
b. Apiculate aleuriospores.

c. Chlamydospore formation, intercalary.

d. Chlamydospores.

Figure 45

Humicola sp. 2



are also unusual for Humicola species and it is thought that this may represent a new species belonging to the genus Humicola. Aleuriospore sizes approximate those of H. fuscoatra va. fuscoatra Fass., but the other morphological features do not agree.

Paecilomyces sp.

Isolated only from the air spora but in relatively significant amounts. This species is thought to approximate Paecilomyces varioti Bainier.

At 40°C on potato dextrose agar growth is relatively rapid and low, velvet-like, light yellow-olive (Ridgway pl.30) colonies are formed, these later become light brownish-olive. Growth on Czapek-Dox agar is slow and colonies are thin, olive-brown with a cream to grey reverse.

Conidiophores are very long, mostly 150-500 μ by 3-4 μ. The long, thin sterigmata or phialides are borne terminally and laterally on short side branches, 10-20 μ by 2.5 μ (see Figure 46). Phialides may be regularly arranged but are more usually divergent; solitary phialides also occur on the conidiophore. They measure 12-25 μ by 1.5-3 μ and are strongly acuminate, producing

Figure 46

Paecilomyces variotii

a. Branches on conidiophore bearing elongated phialides.

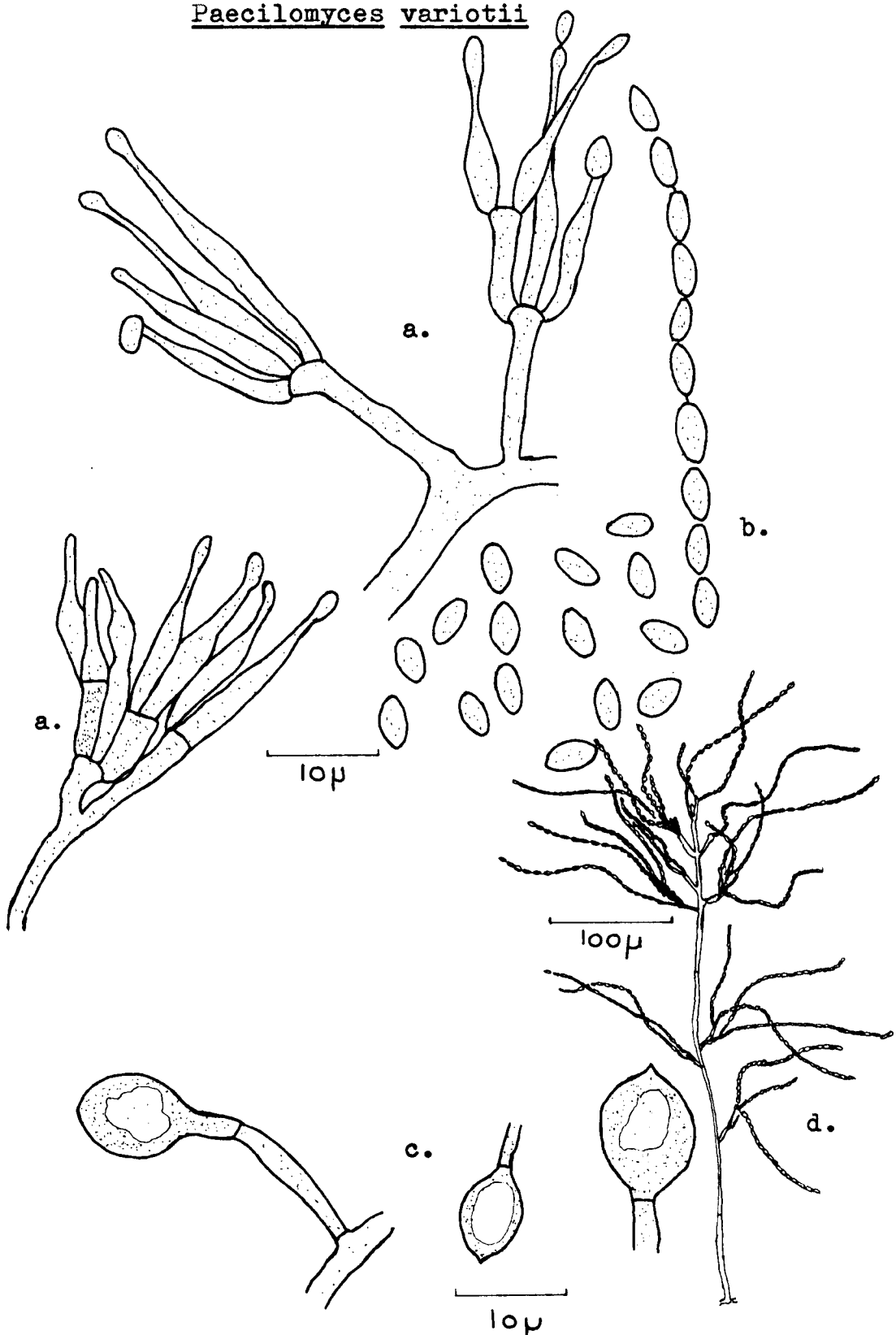
b. Conidia, in chains and separated.

c. Chlamydospores.

d. Habitat view of conidiophore.

Figure 46

Paecilomyces variotii



spores in very long, tangled chains. Conidia are yellow to green in mass, elliptical, mostly 4-4.5(5)μ by 2-2.5μ. Chlamydospores are also formed in the medium arising as side branches on the substrate mycelium. These spores are smooth approximately spherical to globose and often show an apiculate region and dense contents, sizes range for 5-10μ.

Cooney and Emerson (1964, pp.109-110) list several species or synonyms belonging to the genus Paecilomyces and in particular to the P. varioti series. Temperature-growth relationships as given by Sopp (1912) and Cooney and Emerson are very similar to those recorded for the present strains of this Paecilomyces species (see Chapter IV).

Penicillium piceum Raper and Fennell
in Manual of the Penicillia, pp.627-629, 1949.

This species was rarely isolated from a coal spoil tip but was found to be frequent in the air spora.

On potato dextrose agar at 40°C growth is relatively slow and colonies are thin, white and slightly floccose at first but quickly become light green as sporing is initiated. Older colonies usually become dark green or

grey-green depending on the density of spore production. This is a very characteristic species due to the green, fir tree-shaped, penicillial heads, to which the species epithet was applied. Strains were compared with the type species, IMI 40,038, and found to be identical morphologically.

P. piceum was found to be a weakly thermotolerant species and this may explain its involvement in certain animal diseases, i.e. several strains at the Commonwealth Mycological Institute have been isolated from animal sources, such as from cattle infected with mastitis.

Penicillium sp. 1

This species was infrequently isolated from a single coal spoil tip and also from the air spora.

This species could not be traced to any of the described species of Penicillium listed by Raper and Thom (1949) and Kulik (1968). Cultures were sent to Dr. A.C. Stolk at the Centraalbureau voor Schimmelcultures, for identification. Similar strains were already in her possession, being isolated from wood-chip piles in Sweden. Penicillium sp. 1 is considered to be a new species closely related to Penicillium cylindrosporum G. Smith (1957), and a joint paper has

subsequently been written entitled "Penicillium argillaceum sp. n., a thermotolerant Penicillium". The specific epithet refers to the characteristic clay colouration of the colonies when this fungus is grown on a variety of media (see Plate 20a). A condensed version of the paper is given below.

Colonies on Czapek-Doz agar attaining a diameter of 4-4.5 cm. within two weeks at 30°C; with surface growth almost velvety; somewhat loose-textured, consisting of a very thin network of hyphae either above or below the agar surface from which conidial structures arise, in central areas overlaid by a thin overgrowth of loosely interwoven hyphae bearing also penicilli; azonate; with margin irregular in outline, feather-shaped; sporulating somewhat irregularly, showing brownish colours which range from chamois to cinnamon-buff to avellaneous (Ridgway pl. 30, 29, 40); exudate lacking; reverse of colonies uncoloured at first, becoming yellowish or pinkish with age.

Colonies on malt agar spreading broadly, attaining a diameter of 6-6.5 cm. within two weeks at 30°C, in texture and colour as on Czapek-Dox agar but sporulating more abundantly and with margin even, circular in

a. Penicillium sp. 1
7 days on potato dextrose agar at 40°C.

Plate 20

b. Penicillium sp. 2
7 days on potato dextrose agar at 45°C.

outline; reverse uncoloured to avellaneous. Colonies on potato dextrose agar growing rapidly, similar texture and colour as on the other media, with heavy sporulation. At higher temperatures the colonies are characterised by a very strong odour, reminiscent of pine-resin.

Vegetative hyphae hyaline, smooth-walled, 1.5-3 μ in diameter. Conidiophores arising either directly from the substratum or as branches from aerial hyphae, varying greatly in size, ranging from 60-400 μ in length by 2-3(4) μ in diameter, hyaline, septate, with walls usually roughened, but occasionally smooth-walled. Penicilli biverticillate-asymmetric, varying in complexibility, mostly consisting of somewhat appressed verticils of two to five metulae, bearing clusters of phialides, but occasionally developing larger penicilli showing one or two rami in addition to the main axis. Monoverticillate heads are also present. Rami and metulae, as well as metulae and phialides may occur in the same verticil. All elements of the penicillus are hyaline, usually rough-walled, but occasionally smooth-walled. Rami 12-35 x 1.5-3 μ. Metulae 12-25 x 1.5-3 μ, enlarged at the apex to 4 μ in diameter. Phialides 9-14 x 1.5-2 μ, typically occurring in dense clusters

Figure 47

Penicillium argillaceum sp. nov.

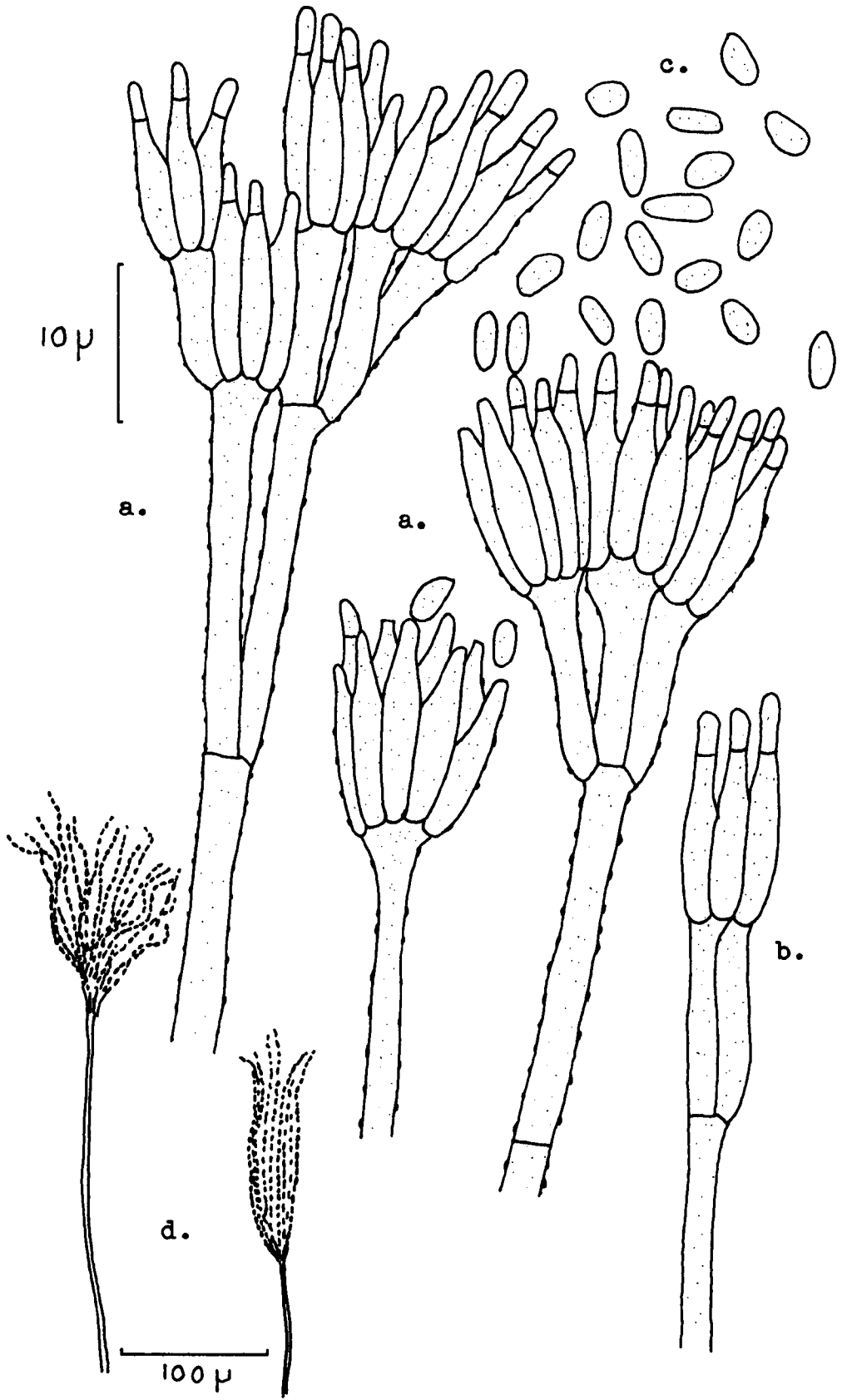
a. Variation in penicilli form.

b. A reduced, smooth-walled penicillus.

c. Conidia.

d. Habitat sketches of penicilli, showing chains of conidia.

Figure 47 Penicillium argillaceum sp. nov.



up to ten in number; consisting of a narrow cylindrical base, tapering more or less abruptly to a narrow conidium-bearing tube, about 1-2 x 1μ. Conidia (2.5)3-4(4.5) x 1.2-2μ, varying from cylindrical to ellipsoid and ovoid, hyaline, smooth-walled. Conidial chains tangled or adhering in loose, twisted columns (see Figure 47).

The type strain is CBS 101.69 isolated from a coal spoil tip with a very high surface temperature, at Leycett, Staffs. in May 1967. A strain isolated from the air spora has also been deposited at the Centraalbureau voor Schimmelcultures, CBS 102.69. This species can be separated from P. cylindrosporum on conidial size, cultural aspects and temperature-growth relationships (see Chapter IV for the temperature-growth relationships of Penicillium argillaceum).

This paper will be published in the Transactions of the British Mycological Society.

Penicillium sp. 2

This species was infrequently isolated from a single coal spoil tip.

On potato dextrose agar at 40°C growth is rapid

and low, white to pearl-grey, strongly funiculose colonies are formed (see Plate 20b). The subterranean mycelium and the colony surface is occasionally yeast-like, often a thick skin or pellicle is formed on the agar surface. Colony margin is entire and with a white or cream reverse. On Czapek-Dox agar growth is slow and hyaline or white colonies are produced. The mycelium is mainly subterranean and no aerial mycelium is present. On malt agar colonies are strongly funiculose and light brown to grey in colour.

The aerial mycelium is aggregated into conspicuous ropes or bundles which project from the agar surface in the forms of spikes, usually enclosed in mucilage. This mycelium is thin, 1.5-2.5μ, hyaline, septate, smooth, and vegetative as penicilli have never been observed on it. Indeed, this species is extremely erratic in its sporulation, forming penicilli only irregularly. However, a series of subcultures made directly from the sporing heads was found to result in the production of more fertile colonies. The penicilli are formed on a separate, low, almost curly, hyaline mycelium, 2-4μ in diameter, and this usually develops around the edges of the colony. On Czapek-Dox agar, very few penicilli are

formed and these arise directly from the substrate mycelium.

Conidiophores are hyaline, erect, septate, short (30-60 μ in length), and usually have a swollen appearance (3-5(6) μ in diameter), smooth-walled. Penicilli are of the Asymmetrica type. These are biverticillate varying in complexity, but mostly consisting of few metulae bearing small clusters of phialides (see Figure 48a). Large penicilli are occasionally produced with one or two rami present in addition to the main axis. Monoverticillate heads also occur (see Figure 48a) and phialides vary in number from one to five. All elements of the penicillus are hyaline and smooth-walled. Rami 10-30 μ by 2.5-3.5 μ; metulae 10-15 μ by 2-4(5) μ, sometimes inflated at the apex. Phialides 10-15 μ by 1.5-2.5(3) μ, usually few in number, consisting of a narrow, cylindrical base tapering fairly abruptly to a narrowed conidium-bearing tube, usually short in length. Conidia 3.5-5 μ (mostly 4.5 μ) by 1.5-2(2.5) μ, varying from cylindrical to ellipsoid and oval, hyaline, smooth-walled. Conidial chains vary in length but rarely exceed 40 μ, chains divergent never dense (see Figure 48). Intercalary chlamydospores also occur in the substrate

Figure 48

Penicillium sp. 2

a. Penicilli; variation in form.

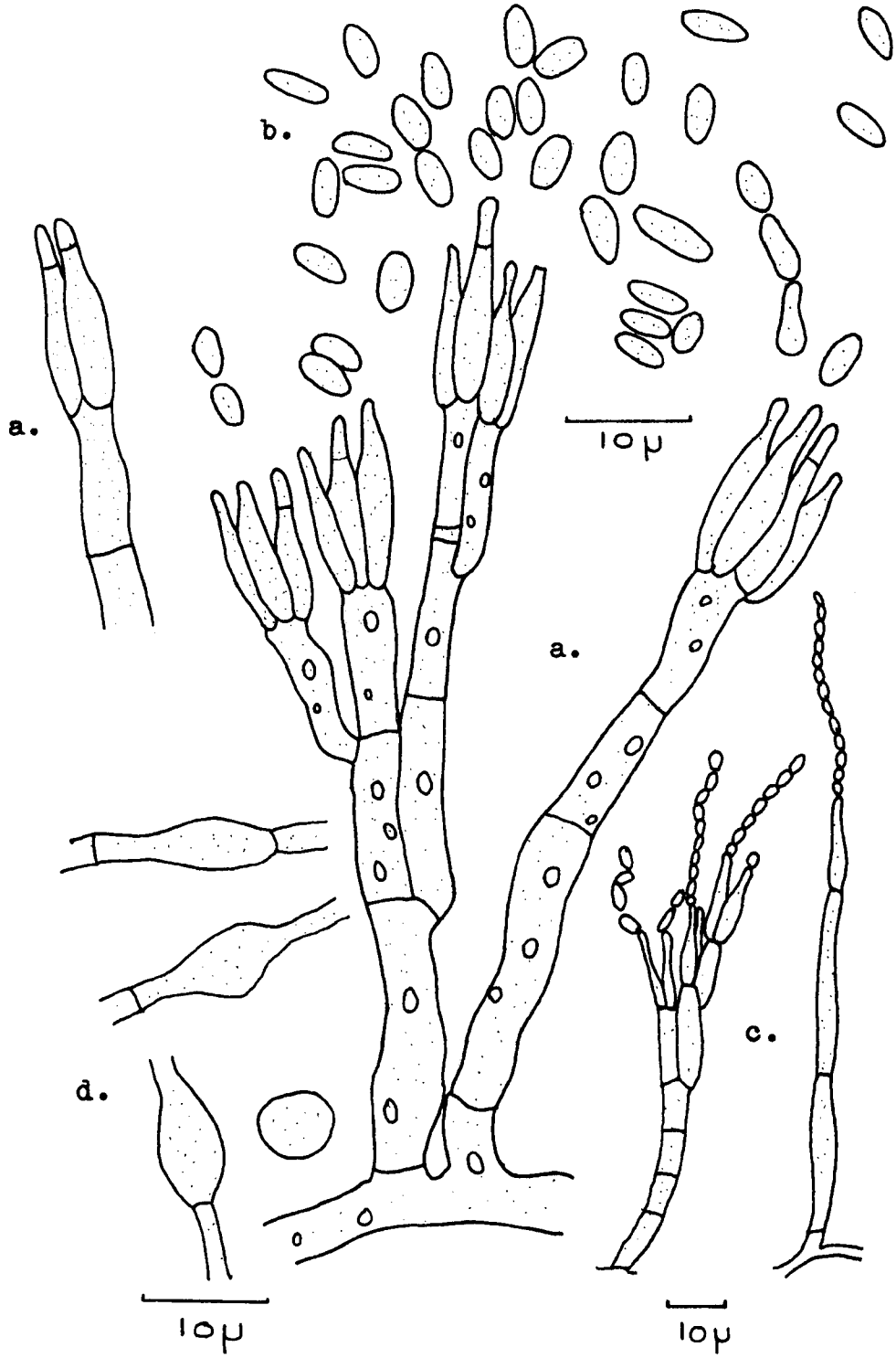
b. Conidia.

c. Habitat view of penicilli, showing chains of conidia and a reduced penicillus bearing a single phialide.

d. Chlamydospores.

Figure 48

Penicillium sp. 2



mycelium, mostly spherical, 5-8 μ in diameter.

Penicillium sp. 2 has penicilli of the P. cylindrosporum, P. argillaceum and P. emersonii type. It is distinct from these species as penicillial heads are never dense and rough-walled elements have never been observed. Furthermore, white-coloured Penicillium species are relatively rare and Penicillium sp. 2 does not approximate any of the known species. Dr. A.C. Stolk considers this to be an undescribed species and it is hoped to describe this species further. The name Penicillium album is provisionally proposed, the specific epithet refers to colony colour. The name P. album has been used before but mis-applied and is not validly accepted, i.e. it is a synonym of P. camemberti Thom, and has also been used to describe white mutant strains (see Raper and Thom 1949). The temperature-growth relationships are discussed in Chapter IV and this species is classified as a true thermophile. The distribution of this species would appear to be very restricted.

Scolecobasidium sp.

This species was isolated exclusively from the warm areas of several coal spoil tips, where it was

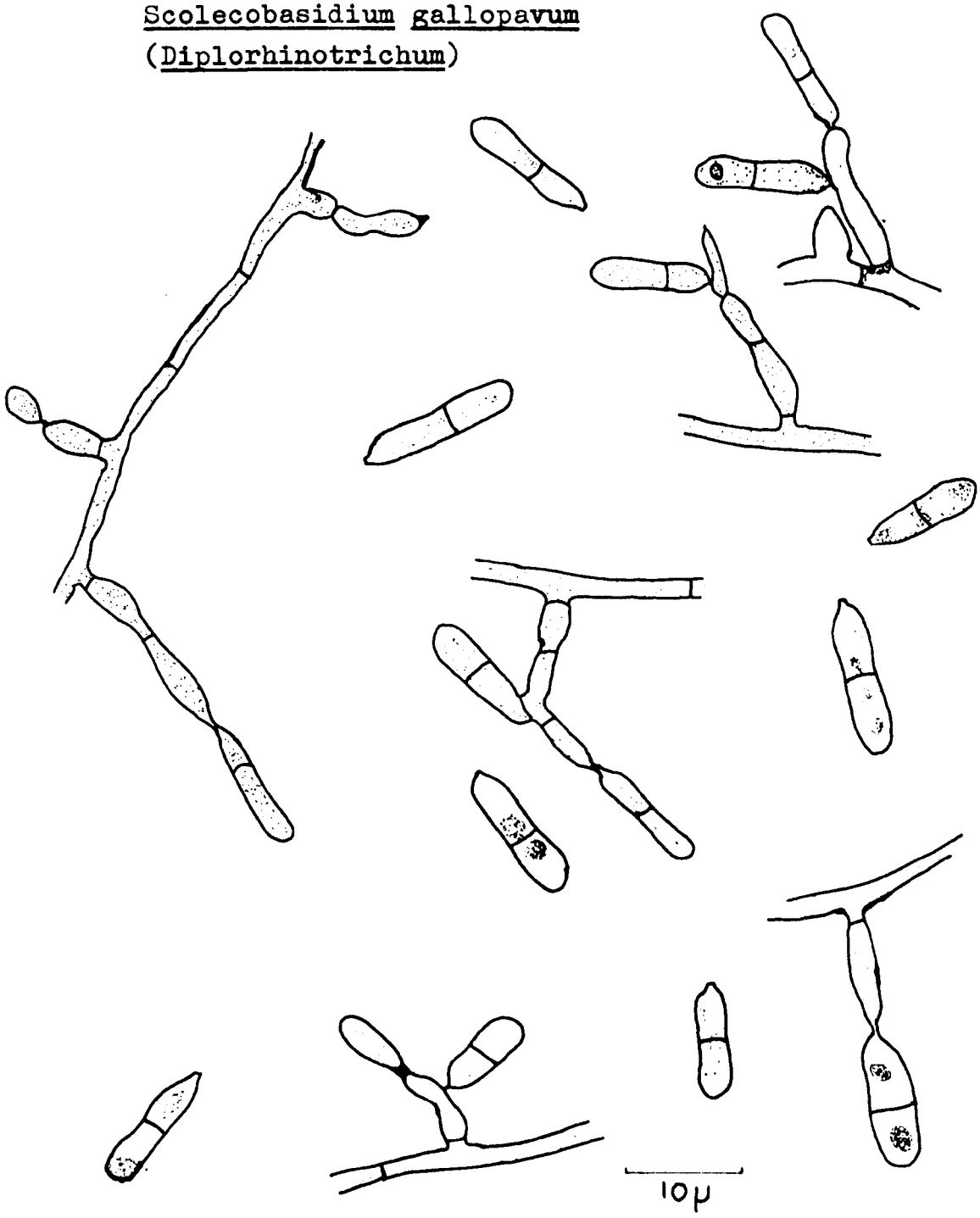
shown to occur in substantial amounts.

Growth on potato dextrose agar and cornmeal agar is relatively rapid at 40°C and low, compact, brown colonies, regular in outline, develop. The turf is generally low but may be raised slightly towards the centre of the colony. Colonies are almost velvet-like and the colour ranges from snuff-brown to olive-brown (Ridgway, pl.29, 40), reverse dark brown. In parts the aerial mycelium may die down, revealing the dark brown substrate or partly subterranean mycelium. The growth pattern is similar on yeast-starch agar and Czapek-Dox agar but the growth rate is markedly reduced (see Plate 21a).

The aerial mycelium is brown, septate, smooth, 1.5-2 μ in diameter. Conidiophores are borne directly from the aerial mycelium or occasionally from the substrate mycelium at the edge of the colony, and are smooth, hyaline to light olivaceous, short and swollen or long and cylindrical and may be bent or twisted at the ends. The range of conidiophore shapes and sizes can be seen in Figure 49, almost spherical or ovoid but more frequently conspicuous conidiophores occur, 3-15 μ by 1.5-3 μ. Conidia borne singly or in clusters, or rarely in acropetal succession from the extended ends

Figure 49

Scolecobasidium gallopavum
(Diplorhinotrichum)



Types of conidiophore structure and variation, showing formation of club-shaped, septate conidia.

of the conidiophores. The sterigmata or denticles on which the conidia are borne may remain attached to the conidiophores or break off forming a tail-piece on the spore. Conidia are smooth, hyaline to pale brown, usually one septate, club-shaped, (8)10-13μ x 2-3μ, occasionally up to 17μ in length, and may appear slightly constricted at the septum.

This species can without doubt be placed in the genus Scolecobasidium as described by Barron and Busch (1962). A search of the literature failed to reveal a similar species belonging to the genus Scolecobasidium. Two strains were sent to Professor Barron and he considers this species as distinct from any of the species of his acquaintance, being closest to S. constrictum. Upon further discussion of this species with Dr. G. Hennebert, who has been studying related species, it was found that a similar species has been recently described but has been placed in the genus Diplorhinotrichum Höhn. This species is D. gallopavum Cooke (see Georg et al. 1964) and it has only previously been isolated from the brain of turkey poults, as the causal agent of encephalitis. No mention is given of the ability of this species to grow at elevated temperatures, although it must

obviously be adapted to the high body temperatures found in warm-blooded animals.

The type strain, CBS 223,59, has been examined and morphologically compares favourably with the present strains. Furthermore, the temperature-growth relaionships are similar, and during preliminary testing the CBS strain grew from 20-50°C, i.e. comparable with the temperature-growth range of Scolecobasidium sp. (see Chapter IV). The high temperature requirements for the growth of this species may explain its infrequent occurrence. This species has never been reported from temperate habitats (normal soils etc.) and it may be that the warm areas of coal spoil tips are conducive to the growth habit of this species.

Dr. Hennebert is about to publish his findings on the taxonomic position of this species and subsequently to transfer it to the genus Scolecobasidium, i.e. S. gallopavum (Cooke) Hennebert, and he has constructed a key to the relevant, related species, viz:

Spores verrucose: Short, not wide - S. constrictum
Long, wide - S. humicola
Spores not verrucose: Long, thin - S. gallopavum

a. Scolecobasidium sp.

Plate 21 7 days on potato dextrose agar at 45°C.

b. Scopulariopsis sp.

Scopulariopsis sp.

This species was isolated from the warm areas of a single spoil tip (Shelton) and it would appear to be of limited distribution.

On potato dextrose agar at 40°C colonies are broadly spreading, initially white to grey, floccose with a cream reverse. After several days dark sectors become evident (see Plate 21b) and with the development of a dark brown stromatic layer the colonies show varying densities of brown areas interspersed with patches of hyaline or grey mycelium, dark brown to black on reverse. On cornmeal agar and yeast-starch agar the development of the brown, mycelial layer is less obvious and colonies appear greyish or creamish-white. On malt agar uneven, grey colonies are produced with a dark brown reverse.

The dark mycelium is roughened, thick-walled, septate, containing dense contents (see Figure 50d), 3-5 μ in diameter and usually erect or semi-erect amongst the hyaline, thin (1.5-3 μ in diameter), aerial mycelium. The phialides or sporogenous cells arise directly from the dark mycelium or more frequently from the hyaline mycelium, either singly or in groups (as reduced verticils). Phialides are hyaline, sessile,

swollen in the middle and tapering to a narrow conidium-bearing tip, mostly 9-14 μ x 3-5 μ, and occasional branching may occur (see Figure 50a). Conidia are borne in long or short chains and are pale brown, ovate to elliptical or fusiform, with pointed ends, 7-10(12) μ x 2.3-4.5(5) μ. The characteristic feature of these spores is the presence of spiral bands which probably represent spines or echinulations, in end view the spores appear unevenly roughened (see Figure 50c).

A similar species was discovered to have been reported by Saksena (1954) and which was further described by Brown and Smith (1957) in their monograph of the Paecilomyces. The name P. fusisporus was applied to this species due to the very conspicuous spore ornamentation. The position was further complicated by the discovery of a report of a similar species, Masoniella indica Salam and Ramarao (1960). However, the genus Masoniella is considered to be invalid (Hughes 1953, Morton and Smith 1963) and thought to be synonymous with the genus Scopulariopsis Bainier, sometimes confused with Paecilomyces. Phialide and conidial morphology of these two species are almost identical and it would appear that M. indica is a synonym of P. fusisporus.

Figure 50

Acrophialophora (Paecilomyces) fusisporus

a. Phialides, showing tapering neck and beginnings of chain formation.

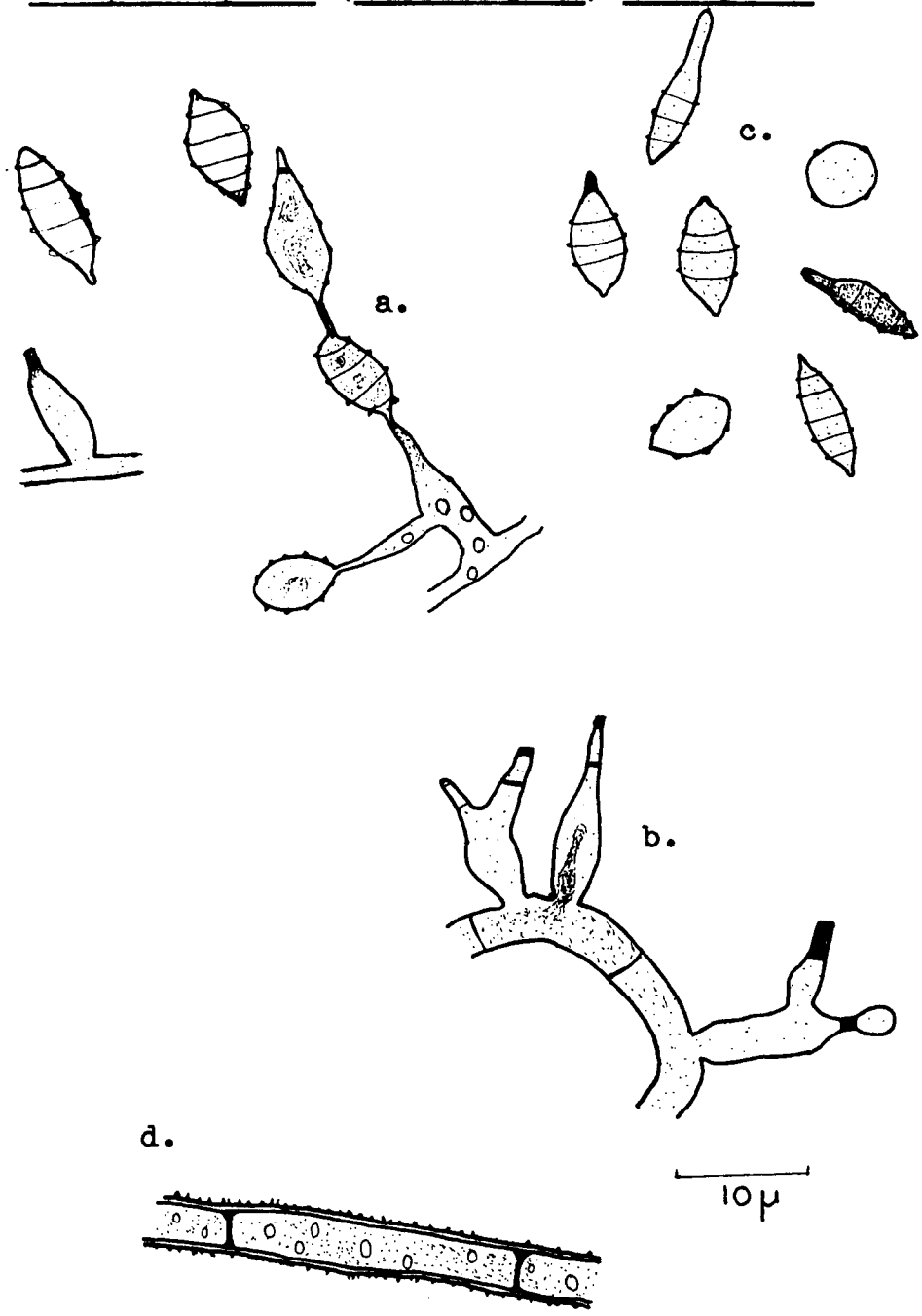
b. Group of phialides, showing branching.

c. Conidia with intricate spiral bands.

d. Portion of errect hyphae; dark, with dense contents and thick, roughened wall.

Figure 50

Acrophialophora (Paecilomyces) fusisporus



A probable third similar species has also been reported by Edward (1959), i.e. Acrophialophora nainiana. Isolates of P. fusisporus CBS 251.55 and A. nainiana CBS 417.67 have been examined and found to compare favourably in morphological detail with the present isolates labelled Scopulariopsis sp. It must be assumed that M. indica is also a strain of the same species.

P. fusisporus does not readily produce the dark, roughened mycelium and colonies are usually light brown in colour; However phialide and spore morphology are identical with the other species (strains). Edward observed definite conidiophores in his strains and these constitute the erect, thick-walled, roughened hyphae which arise from a basal hyphal cell and bear phialides only at the upper end. On the basis of the conidiophore morphology he erected the genus Acrophialophora as distinct from the genera Paecilomyces and Scopulariopsis. The author states, however, that sessile phialides occurring on the creeping or aerial mycelium (hyaline) are more common in young cultures and the present strains develop this state much more readily than the conidiophore state. It was reported above (see

diagnosis) that phialides are found on the erect, dark mycelium and this may represent the reduced conidiophore stage. These dark hyphae are described by Salam and Ramarao but were thought to constitute the vegetative mycelium, their cultures must obviously produce phialides more readily on the hyaline, creeping mycelium. The latter authors state a slow growth rate of their isolates at room temperature whereas Saksena reports P. fusisporus as having a medium growth rate at this temperature. Only Edward mentions the ability of this species to grow at higher temperatures and he gives an optimum of 32°C for his strains and indeed this species was frequently isolated during the warm summer months in India. An examination of A. nainiana revealed that this species is able to grow well up to 45°C, although growth is hardly perceptible at higher temperatures (50°C), this is almost comparable with the results obtained for the temperature-growth relationships of the present strains (see Chapter IV).

The elaborate, spiral markings on the spores were not commented upon by Edward, although he recorded that they may be slightly echinulate, but distinct ornamentations were noted by the other authors. These

markings are, however, very much more apparent in the present strains (Scopulariopsis sp.) than any of the other strains examined, the latter may be termed irregularly echinulate. This species has previously only been reported from India and it is interesting to note that this may be a direct result of its high temperature-growth requirements. Due to its very slow growth at normal isolation temperatures it may have previously been over-looked, especially in temperate climates where soil temperatures are rarely sufficiently high to encourage optimum development of this species. The warm areas of coal spoil tips would appear to be a suitable habitat for the development of this species.

Dr. Hennerbert considers that all these strains are distinct from the genus Paecilomyces (and indeed from the genus Scopulariopsis) and should be included in the genus Acrophialophora under the new combination Acrophialophora fusisporus.

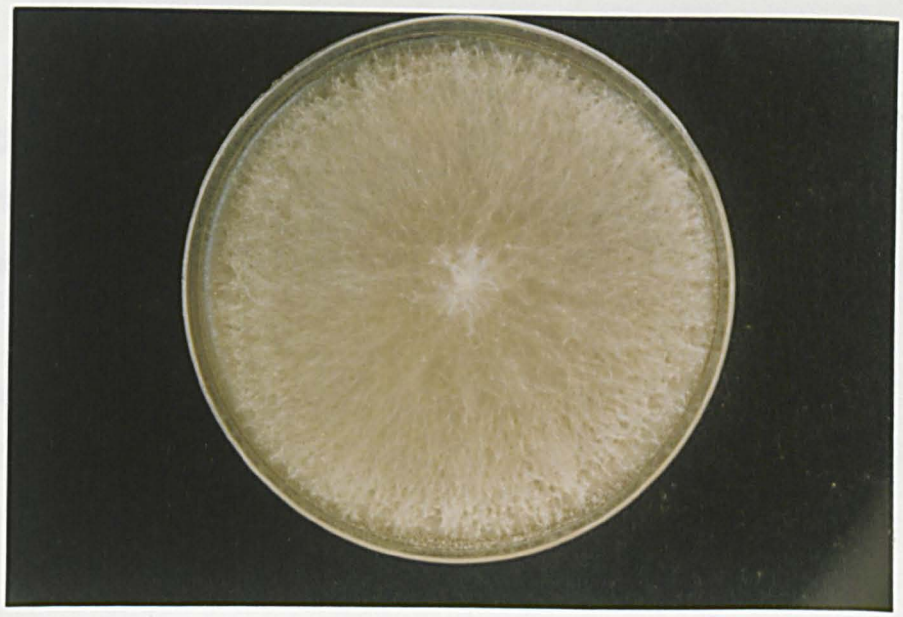
Sporotrichum thermophile Apinis
Nova Hedwigia 5, p.74, 1963.

Sporotrichum thermophile was isolated from a number of habitats during the present study and would appear to

a. Normal strain

Plate 22 Sporotrichum thermophile
7 days on potato dextrose agar at 40°C.

b. Green strain



be ubiquitous.

On potato dextrose agar at 40°C growth is extremely rapid and dense, white, floccose colonies are initially produced. These become pink after several days and eventually assume various shades of orange or brown (see Plate 22a). At lower temperatures in particular, dark brown or orange brown colonies result due to heavy sporulation. Colony colour, however, was found to be variable and green-coloured strains were occasionally isolated (see Plate 22b). The conidia are usually smooth but rough-walled spores may also be present. In other morphological details the strains agree well with the described species.

Sporotrichum sp.

This species was isolated only from the air spora. It is now considered to be a member of the genus Chrysosporium and approximates C. lutem (Costantin) Carmichael. A strain of this species (CBS 227.67) was examined and agreed well with the present strains.

Growth on all media is slow at 40°C and wrinkled, lime-green, yellow to dirty brown colonies are formed with a dark brown reverse. The margin is irregular and

Figure 51

Chrysosporium luteum

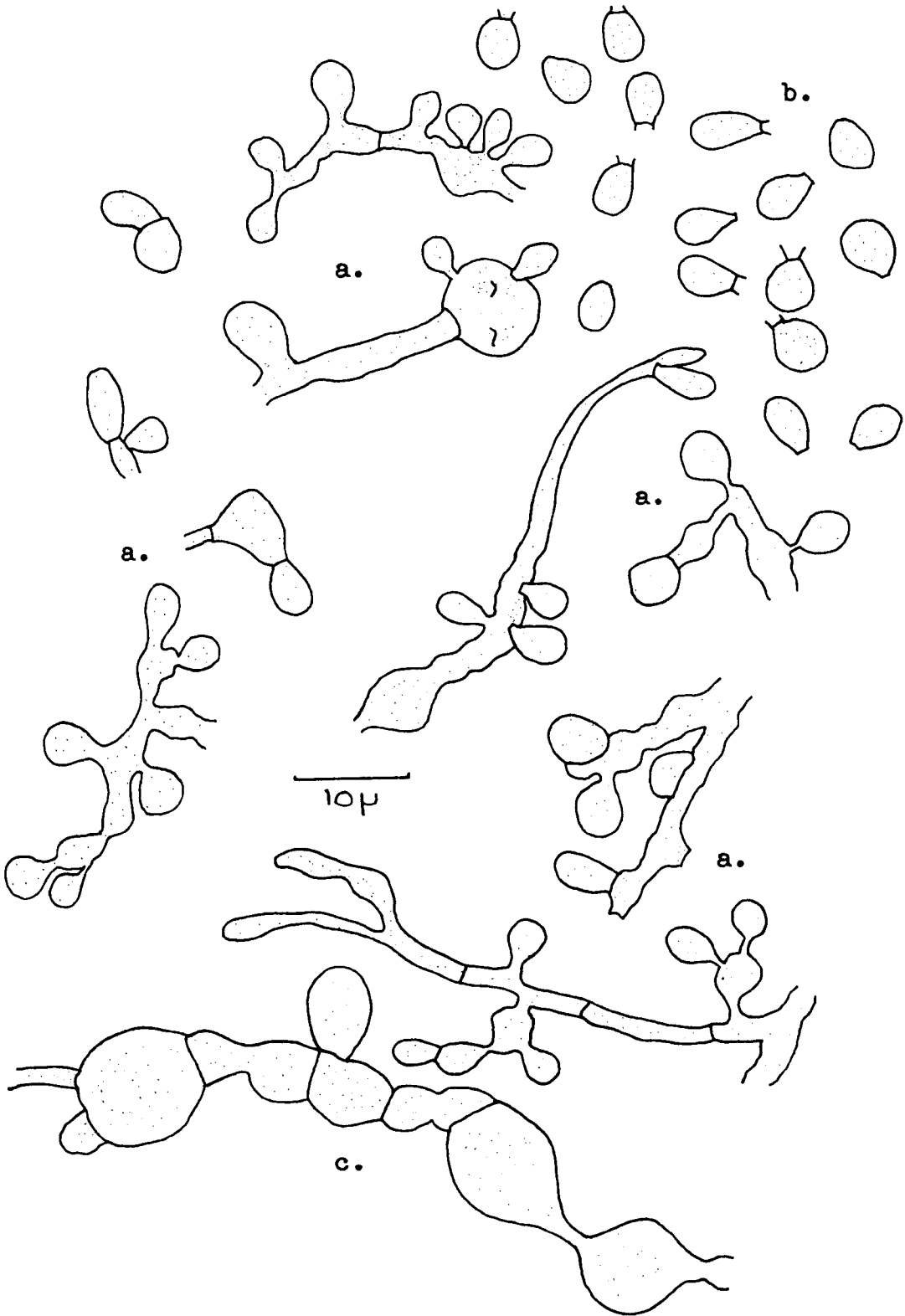
a. Types of spore formation.

b. Conidia.

c. Chlamydospores.

Figure 51

Chrysosporium luteum



the colonies appear powdery in the centre. A brown diffusate is sometimes noticeable. The aleuriospores are borne directly on the hyphae, or on short pedicels, or on ampulliform swellings (see Figure 51) as described by Carmichael (1962, pp.1158-1159). Spores are spherical to globose, frequently with a truncate portion and vary from 3-4 x 4-6μ. Large swellings occur in chains along the mycelium and it is thought that these are chlamydospores, they usually occur in older cultures and vary from 10-15μ. The temperature-growth relationships of this species have not been accurately determined but the range is about 15-46°C with an optimum at approximately 37°C. It is, therefore, in no way thermophilic and in fact is only weakly thermotolerant. Most previous strains have been isolated from mushroom beds and is the cause of 'mat' disease of commercial mushrooms.

Thermoidium sulphureum Miehe
Ber deutsch bot. Gesl., 25, 510-515, 1907.

This species was re-described by Cooney and Emerson (1964) who transferred it to the genus Malbranchea, originally described by Saccardo 1882, under the name

M. pulchella var. sulphurea. In the present context this species is designated Thermoidium sulphureum as under this name the first valid and comprehensive description of this species was published by Miehe.

The characteristic and striking sulphur yellow colour of this species is produced on a variety of media and is often preceeded by a pinkish colouration developing in the marginal mycelium. A red diffusate is occasionally noticeable in the agar and the colony reverse may appear wine-red. This proved to be a fairly widespread organism, although it was never isolated in significant amounts.

The morphology of Thermoidium sulphureum is as described by Cooney and Emerson and other spore stages have never been observed. Dr. G. Hennebert (personal communication) has considered transferring this species to the genus Geotrichum due to the production of arthrospores from the terminal portions of the vegetative hyphae.

Thermomyces lanuginosus Tsiklinsky

Sur les mucédinées thermophiles. Ann. Inst. Pasteur 13, 502, 1899.

A detailed account of the genus Thermomyces origin-

-ally erected by Tsiklinsky (1899), is given by Pugh et al. (1964) and reports on the synonyms of T. lanuginosus are discussed by Apinis (1963a, p.75) and Cooney and Emerson (1964, pp.82-83). The latter authors accept the transference of this species to the genus Humicola on the basis that T. lanuginosus was incompletely described by Tsiklinsky. The inclusion of this species in the genus Humicola was originally proposed by Bunce (1961). However, the original name is accepted here due to the elucidation of this genus by Apinis (1963a) and by Pugh et al. (1964).

The former author transferred Humicola stellatus Bunce (see Cooney and Emerson 1964, pp.79-82) to the genus Thermomyces because of the absence of phialospores and the presence of stellate aleuriospores which are similarly formed to those of T. lanuginosus. Since then several new species have been added to the genus Thermomyces and only one species has failed to show thermophilic tendencies. T. verrucosus Pugh, Blakeman and Morgan-Jones (1964) is described as mesophilic in temperature relationships but T. ibadanensis Apinis and Eggins (1966) is considered to be a true thermophile. The latter species is distinguishable by its smooth,

spherical conidia.

Growth of T. lanuginosus is rapid at 45°C on a variety of media and a considerable variation was found to occur amongst the strains isolated during the present study. On potato dextrose agar the fungus may vary from bushy, grey colonies to dark green, low colonies, and growth on the agar surface is often reminiscent of yeast or bacterial colonies. On yeast-starch agar colonies are pinkish-grey, floccose, with conspicuous red exudate droplets, often a diffusate stains the medium a dark red colour.

Morphologically the present strains approximate those reported by the previous workers. This species was the most frequently isolated of the true thermophiles and was ubiquitous in its distribution. This may be due, in part, to rapid spore production during favourable conditions and the possibility that the thick-walled spores are able to survive for long periods.

Tritirachium sp.

This species was rarely isolated from a single coal spoil tip.

On yeast-starch agar and Czapek-Dox agar at 40°C

a. Tritirachium sp.

Plate 23 7 days on potato dextrose agar at 45°C.

b. Aspergillus fumigatus:
Green strain with orange mutant sector.

growth is relatively slow, faster on potato dextrose agar and malt agar. Colonies regular in outline, compact almost velvet-like, light pinkish-cinnamon to avellaneous (Ridgway, pl. 29, 40) (see text Plate 23a), with a light to dark brown reverse. Sporing is very heavy and little vegetative mycelium is present, colonies occasionally appear bush-like. The colonies emit a distinct, sweet, spicy odour and this is particularly noticeable at high temperatures.

Conidiophores are upright, smooth, septate, light brown, single or giving rise to primary and secondary branches (see Figure 52c), 100-300 μ long occasionally up to 400 μ, 1.5-3 μ wide. Conidia are borne in acropetal succession from swollen areas near the apex of the conidiophore. They arise on conspicuous denticles which often remain as prominent scars after the spores have been shed. The continued proliferation of the conidiophore gives rise to a succession of swellings which are often formed in an orderly arrangement, almost rachis-like. This regular conidiophore proliferation imparts a reduced Tritirachium-like appearance to the conidiophores, but this is only evident in old cultures. Small, irregular branches may develop from the apical

Figure 52

Tritirachium sp.

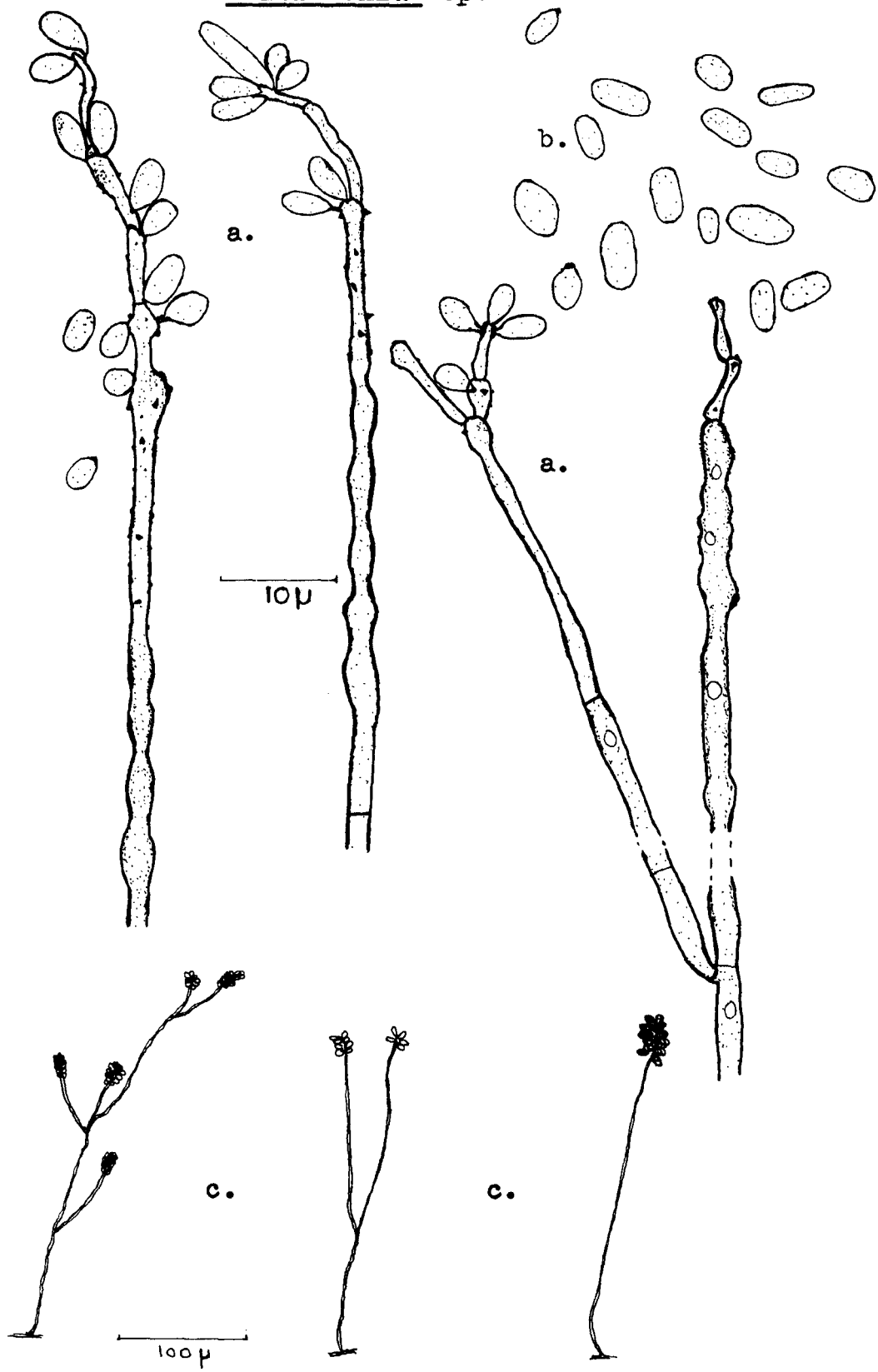
a. Apex of conidiophores showing conidia formed on denticles arising primarily from swollen regions.

b. Conidia.

c. Conidiophore habit, showing variation in branching.

Figure 52

Tritirachium sp.



swellings. The conidiophore wall immediately below the sporogenous areas is occasionally roughened and this may be due to the old scars of conidial production. Conidia smooth, pale yellow, oblong to oval, occasionally apiculate, 4-6(7) x (1.5)2-3 μ.

The cultural characteristics of this species approach those of the genus Tritirachium and an examination of a culture of T. cinnamomeum Van Beyma (CBS 182.42) revealed very similar growth patterns. However, the lack of a true verticillate arrangement of the sporogenous cells and of an exaggerated rachis-like formation by the present species, diagnostic features of the genus Tritirachium (see Limber 1940, Van Beyma 1942, Mehrotra and Basu 1967), led me to believe that this species should be more correctly placed in a separate genus closely related to Tritirachium. The formation of conidia on denticles arising from conspicuous swellings on the conidiophore is characteristic of this species.

Cultures were examined by Dr. G. Hennebert who assigned them to a separate, as yet un-named, genus, which shows affinities with several other genera, viz: Tritirachium, Geniculosporium Chesters and Greenhalgh, and Nodulosporium Preuss. Dr. Hennebert has decided to

study these isolates further and hence the taxonomic position of this species is as yet uncertain.

CHAPTER VI

CELLULOLYTIC ABILITY

OF

THERMOPHILOUS FUNGI

Introduction

The investigation of the cellulolytic ability of the fungi isolated during the present study was made in order to test the importance of these fungi as colonisers of leaf-litter, humus and composts in general, and hence to discover what part they might play in the initial degradation of plant materials. In fact, the preliminary aim of the present thesis was to study the cellulolytic fungal species which develop at high temperatures. In order to achieve this aim, samples were incubated on Warcup soil plates with a medium of cellulose acetate or ethyl cellulose as the carbohydrate source plus a mineral salts agar. As a result of this method the following species were isolated from manure and garden soil: Aspergillus fumigatus, Thermoidium sulphureum, Talaromyces duponti and Talaromyces emersonii. Another medium containing cellophane (25 gms/litre) plus a mineral salts agar was tested and the following species were isolated from grass cuttings and compost: Aspergillus fumigatus, Thermoidium sulphureum, Humicola insolens, Torula thermophila, Myriococcum albomyces, Chaetomium thermophile

and Talaromyces duponti. The success of these preliminary isolation methods and the apparent ubiquitous distribution of these thermophilic fungi stimulated the writer to broaden the study of thermophilic fungi and thus to concentrate more on an ecological and taxonomic approach. However, it was still decided to test the cellulolytic ability of all the species of fungi isolated during the ecological studies.

Information concerning the cellulolytic ability of thermophilic fungi is relatively scarce. Isachenko and Mal'Chevskaya (1936) reported that there was extensive cellulose breakdown in self-heating peat piles. They failed to isolate any thermophilic fungi which showed active cellulolytic properties and found that Talaromyces duponti grew poorly on cellulose only weakly attacking it, whereas Thermoascus aurantiacus did not attack cellulose at all. Waksman et al. (1939b), working probably with Humicola insolens, (see Cooney and Emerson 1964, p.130) found that after two days at 50°C the cellulose content of stable manure dropped from 19.7% to 12.6%. Waksman and Cordon (1939) reported similar results, this time using straw and alfalfa residues. Humicola insolens was proved conclusively

to be an active cellulose decomposer by several later workers (Reese 1946, Henssen 1957 and Chang 1967). Henssen (1957) found that a thermophilic Sporotrichum sp. (S. thermophile) was able to grow weakly on nutrient agar with pure cellulose as the carbon source but that Thermomyces lanuginosus showed no growth at all. The inability of T. lanuginosus to attack cellulose has also been reported by Reese (1946) and Chang (1967). The latter author tested the cellulolytic ability of a number of thermophilic fungi isolated from wheat straw compost and found that Mucor pusillus, Talaromyces duponti and Thermomyces lanuginosus were unable to utilise cellulose. Thermoidium sulphureum was found to be scarcely able to use it but Humicola insolens, Sporotrichum thermophile and Chaetomium thermophile were reported to be strongly cellulolytic. Chaetomium thermophile was able to break down much more cellulose than any other fungus tested over the three week incubation period. Aspergillus fumigatus was also reported as being a strong cellulose decomposer confirming the classification by Siu (1951) who included this species in the group comprising the very active cellulose decomposers.

Methods

The initial aim was to measure colony growth on a cellulose agar medium and to compare this with weight loss when grown on filter paper plus a mineral salts solution (Garrett 1962). However, the cellulose agar medium proved to be unsuccessful as an accurate measure of colony growth, due to the fact that non-cellulolytic fungi (e.g. Phycomycetes) were able to colonise the plates rapidly during the first few days, seemingly without actually utilising the cellulose but probably by metabolising food reserves and impurities present in the agar. To elucidate this problem, fungi were inoculated onto a mineral salts agar with finely chopped filter paper as the sole carbon source and a control experiment was run in which no carbon source was added to the mineral salts agar. Most of the species tested were able to grow on the cellulose agar but were also able to grow, almost equally as well in some cases, on the agar medium alone. In the majority of cases growth was not as dense on the latter medium and usually lasted only several days but accurate measurement of colony growth was considered to be impossible.

Finally two methods were selected to measure the

cellulolytic ability of thermotolerant and thermophilic fungi:

Method i. By measuring cellulase activity.

Method ii. By measuring loss of weight of cellulose.

i. Cellulase Activity

An acceptable method has recently been reported by Savory et al. (1967). The method is based on that first proposed by Eggins and Pugh (1962) who used mineral agar containing 1% ball-milled Whatman cellulose as the sole carbon source. The appearance of a clearance zone in the opaque medium around an inoculum is taken to indicate cellulase production. Savory et al. improved upon this method by first growing the fungus on a cellulose/mineral agar plate and then transferring a plug of the fungus plus medium to a recess in a new plate of the same medium. The physical check to growth of the fungus caused by cutting the plug enabled the enzymes from it to diffuse into the surrounding test medium, resulting in a well defined clearance zone if cellulase was present. In order to prevent the fungus from growing over the medium and thereby destroying the clearance zone, sodium azide was included in the test medium. The sodium azide acts as a respiratory inhib-

-itor preventing growth of the fungus from the plug but not affecting the outward diffusion of cellulase, if present. Thus, by measuring the clearance zones produced, if any, a comparative analysis of the cellulolytic ability of a range of fungi can be undertaken.

Material: Mineral agar composition

Ammonium sulphate	0.5 gm.
Potassium dihydrogen phosphate	1.0 gm.
Potassium chloride	0.5 gm.
Calcium chloride	0.1 gm.
Crystalline magnesium sulphate	0.2 gm.
Yeast extract	0.5 gm.
L - asparagine	1.0 gm.
Oxoid agar No.3	20 gm.
Distilled water	1 litre

pH adjusted to 6.2

In the cellulose/mineral agar medium the 1% ball-milled cellulose was replaced by Whatman Chromedia cellulose powder (mean particle size 15-40 μ). The cellulose powder is a very convenient form of cellulose as no preparation is involved, in contrast to the ball-milled cellulose which involves laborious processing, and a very homogenous medium is obtained.

Ten gm. of the cellulose powder and 0.325 gm. of sodium azide were added per litre of mineral agar. The plugs were taken with a stainless steel cork borer as sodium azide is liable to dissolve the copper in a normal borer. The radial width of the clear zone was measured, when possible, using transmitted light.

Results

The results with sodium azide were very disappointing due to the fact that the majority of fungi appeared to be completely inhibited by this compound and cellulase activity was not detected in the area immediately around the inoculum plug, i.e. no clearance zones or rings were produced. Sodium azide was, therefore, excluded from the test medium and reasonable results obtained thereby. The majority of species, in fact, did manage to grow on the cellulose agar despite their obvious inability to utilise cellulose as a carbon source, and for these species growth must be supplemented by other carbon sources present in the medium or as food reserves in the mycelium.

The thermophilous species can be classified into four groups on the basis of their ability to clear

cellulose powder agar, viz:

Group 1: those species which failed to clear cellulose agar but which, in general, grew well on this medium.

Group 2: those species which produced faint clearance of the medium around the inoculum plug, but for which clearance zones were not sufficiently developed to be measured.

Group 3: those species which produced definite, measurable clearance zones (rings) in the medium.

Group 4: those species which grew rapidly over the medium and which did not produce measurable clearance zones, but which cleared the cellulose medium over a wide area of the plate.

Group 1

A large number of the fungi tested were unable to clear the cellulose powder but were able to grow on the agar medium. These species must utilise the small amounts of asparagine, yeast extract and impurities present in the medium.

Species include:

Aspergillus spp. 1, 2 and 3 Rhizopus spp. 1, 2 and 3

Dactylomyces crustaceus

Dactylomyces thermophilus

Geotrichum sp.

Mortierella sp.

Mucor pusillus

Paecilomyces sp.

Penicillium piceum

Penicillium spp. 1 and 2

Scolecobasidium sp.

Talaromyces duponti

Talaromyces emersonii

Talaromyces sp.

Thermoascus aurantiacus

Thermomyces lanuginosus

Tritirachium sp.

Hyphomycete sp. (Calcarisporium)

The extremely rapid growth of some of these species (e.g. Phycomycetes) may be of tremendous survival value in natural habitats, i.e. the ability to grow rapidly over appreciable distances utilising a minimum amount of food material. Dactylomyces thermophilus, Talaromyces emersonii, Talaromyces sp., Thermoascus aurantiacus all produced ascocarps abundantly, and this may have considerable survival value when food supplies are limiting.

Group 2

These fungi can be considered to be weak cellulose decomposers, possessing a small amount of cellulase activity. For these species, slight clearance zones

were produced around the inoculum plug where cellulase activity was most intense.

Species include:

Chaetomium sp. 1 (Thielavia)

Sporotrichum sp. 1 (C. luteum)

Scopulariopsis sp. (Acrophialophora)

Thermoidium sulphureum

Trichophaea sp. (Sphaerospora)

For Chaetomium sp. 1 and Trichophaea sp. growth was fairly dense, but only a very thin ring was present around the inoculum plug. Plate 24 shows this faint ring around the plug of Chaetomium sp. 1, cellulose powder particles have been removed by cellulase activity from this area.

Sporotrichum sp. 1 produced a dark brown exudate which stained the medium. Thermoidium sulphureum formed an extremely dense growth and produced a pinkish-yellow diffusate in abundance. However, no measurable rings were apparent.

Group 3

This group includes species of differing cellulolytic ability shown by the range of size of clearance zones produced. Clearance of the medium indicates cellulase

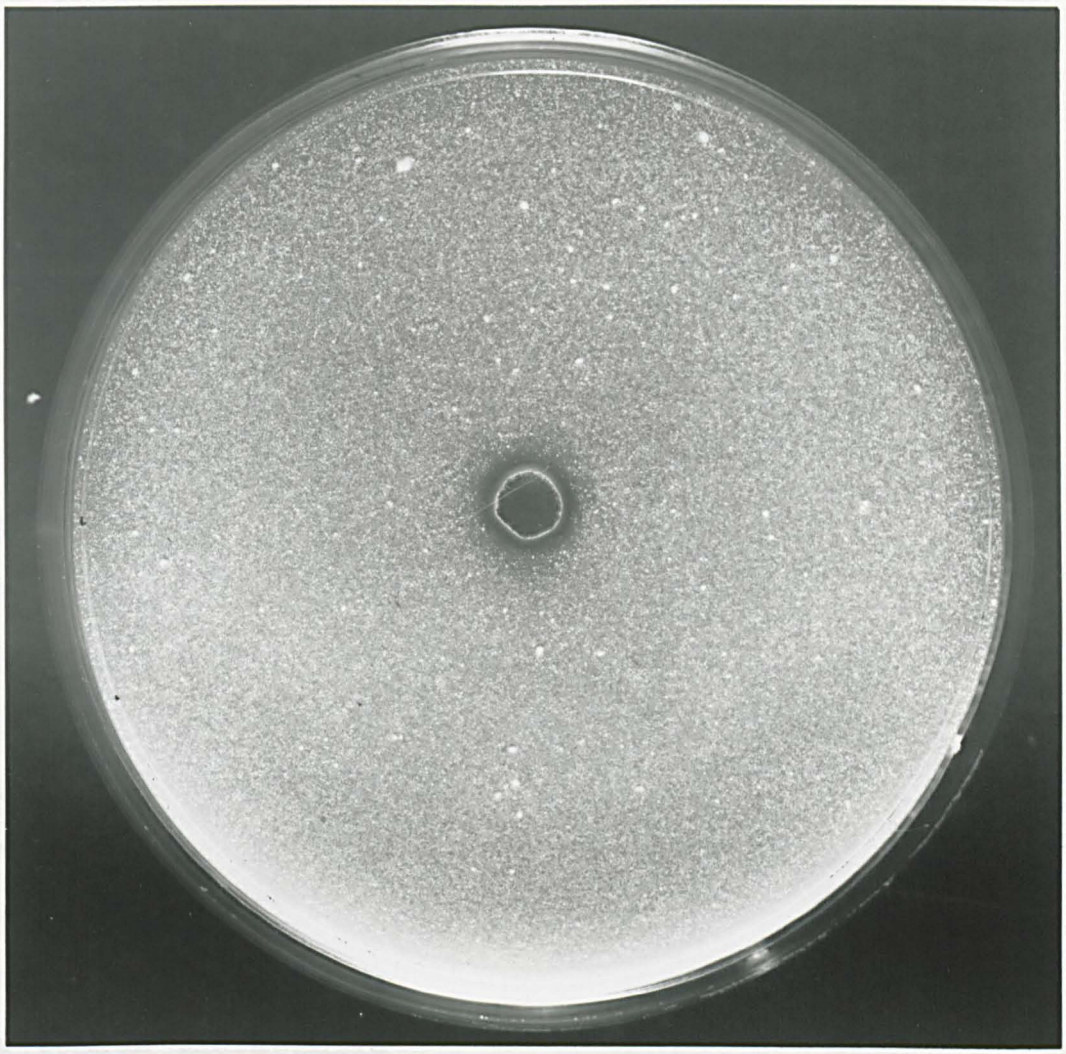


Plate 24

Clearance zone in cellulase assay plate (x 1.5)

Produced by insertion of a plug of agar cut from another plate of Chaetomium sp. 1

21 days incubation at 40°C.

activity and the extent of this clearance will be determined by the relative amounts of cellulase produced. Hence, this will give a measure of cellulase activity and, therefore, cellulolytic ability.

The species are listed in order of the size of clearance zones produced, i.e. cellulolytic ability.

Species or strain	Size of ring (radius) in mms.
Allescheria terrestris st. 1	2
Allescheria terrestris st. 2	3
Chrysosporium sp. 1	3
Chrysosporium sp. 2	4
Cephalosporium sp. 2 (Phialophora)	4
Cephalosporium sp. 1	5
Aspergillus nidulans	5
Chaetomium sp. 2	6
Humicola sp. 1	6
Humicola sp. 2	7
Chaetomium thermophile	9
Sporotrichum thermophile st. 1	8.5
Sporotrichum thermophile st. 2	10
Aspergillus fumigatus, olive var.	10

These results are compiled from an average of four

plates after three weeks growth at 40°C or 45°C.

Both strains of Allescheria terrestris produced numerous, mature perithecia on this medium, whereas most other media failed to encourage these stages. Also Chrysosporium sp. 2 and Cephalosporium sp. 2 produced ascocarps in varying degrees of abundance on this cellulose medium.

Chaetomium thermophile, Sporotrichum thermophile and Aspergillus fumigatus (olive var.) would appear to be the strongest cellulose decomposers of this group. The clearance zones produced by the former two species can be seen in Plates 25 and 26 respectively. The photographs were taken of the lower surface of the petri plate due to extensive colony growth on the upper surface which masks the clearance rings.

Group 4

These species can be considered to be strong cellulose decomposers due to the extensive clearance of the cellulose powder. The rapid growth of these species may produce a general spread of cellulase activity and specific zones or areas of intensive cellulase activity are not apparent.

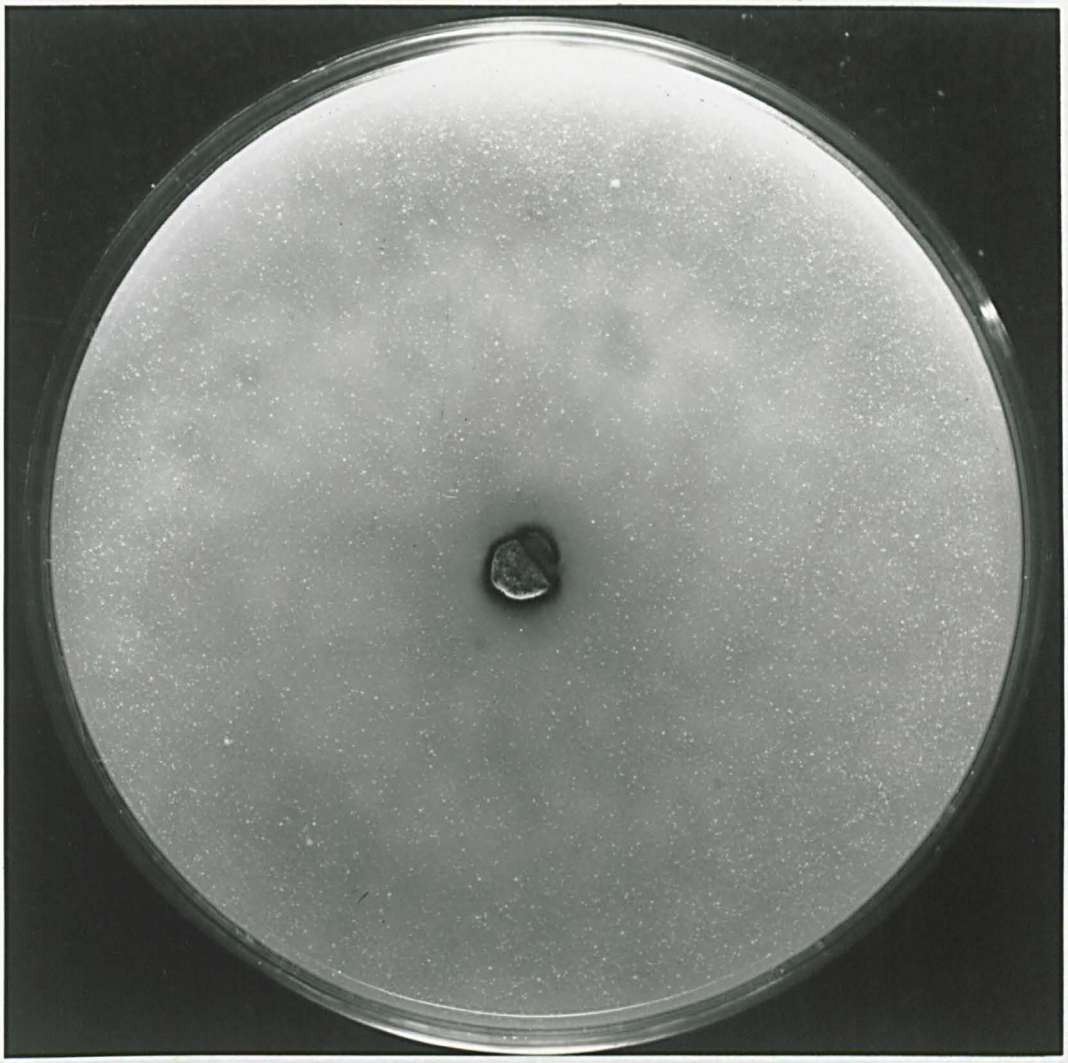


Plate 25 Lower Surface

Clearance zone in cellulase assay plate (x 1.5)

Chaetomium thermophile

14 days incubation at 45°C.

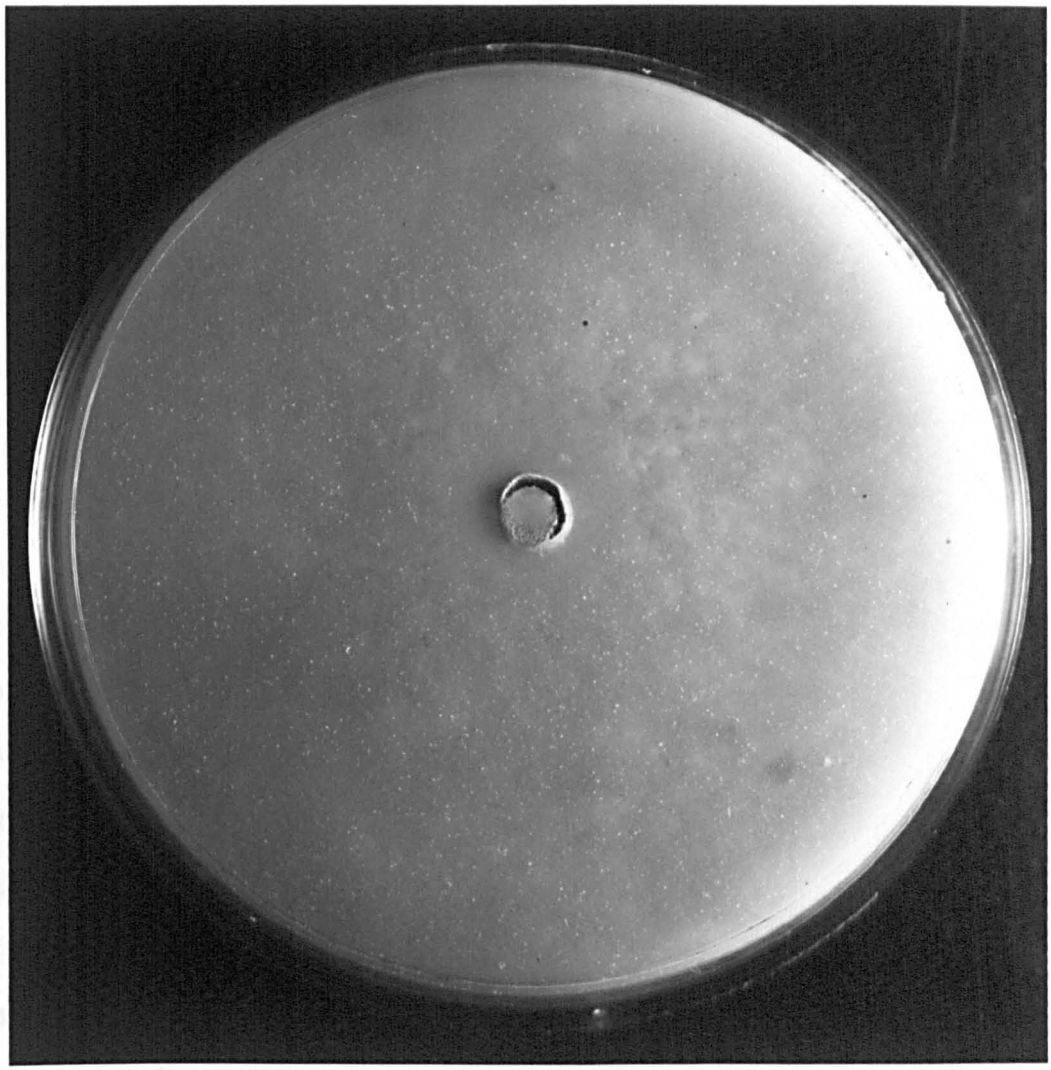


Plate 26 Lower Surface

Clearance zone in cellulase assay plate (x 1.5)

Sporotrichum thermophile

14 days incubation at 40°C.

Species include:

Aspergillus fumigatus	Myriococcum albomyces
A. fumigatus (orange var.)	Torula thermophila
Humicola insolens	

Humicola insolens and Torula thermophila produced a dense subterranean growth as well as a heavy surface growth. Myriococcum albomyces produced abundant cleistothecia on this medium. However, the clearance of the medium by these species was not as marked as that produced by the orange variety of Aspergillus fumigatus which would appear to be a very strong cellulose decomposer. Plate 27 shows this species after a few days growth at 40°C, a clearance zone has appeared around the inoculum plug but the cellulase activity is becoming more general and the medium is being cleared over a large irregular area which will eventually include the whole plate. Aggregations of cellulose particles can be discerned at the edges of this irregular clearance zone.

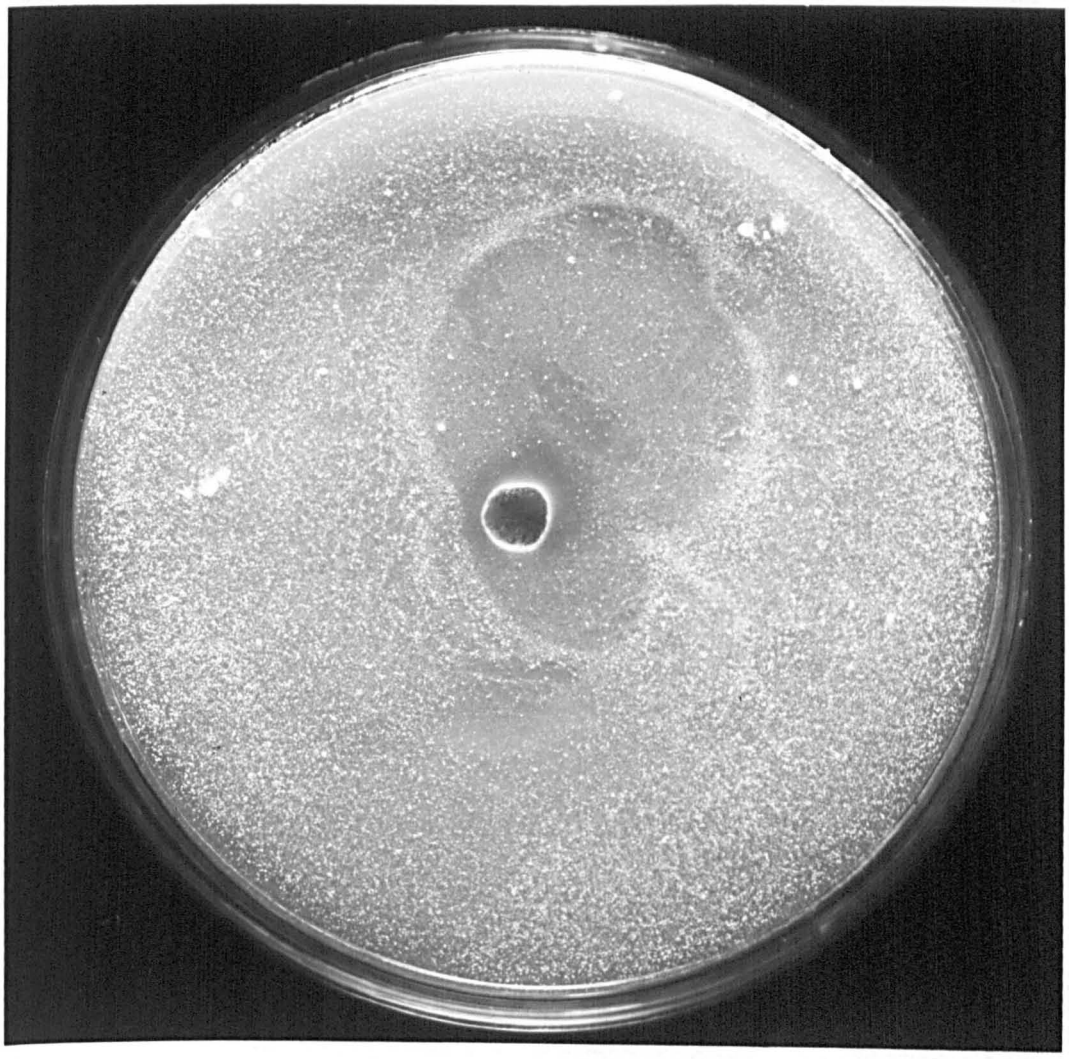


Plate 27 Lower Surface

Irregular clearance of cellulase assay plate (x 1.5)

Aspergillus fumigatus

(Orange var.)

4 days at 40°C

ii. Loss of weight

The method employed was essentially the same as that described by Garrett (1962). Wads of ten filter papers (Whatman No. 3) were oven-dried, weighed and placed in 150 ml. conical flasks. 30 ml. aliquots of a mineral salts solution were added and the flasks autoclaved for twenty minutes at 15 lb/in^2, the original aliquot used was 15 ml. as suggested by Garrett but desiccation occurred over the three week incubation period at elevated temperatures. Each flask was inoculated with a 6 mm. disk from an actively growing colony and control flasks were set up using disks of blank agar as inocula. Incubation was carried out at 40^{o}C but for comparative reasons a few selected species were grown at lower or higher temperatures. The filter pads were then removed and oven-dried at 80-100^{o}C and re-weighed to constant weight. During the weighing procedure great care was taken to reduce the amount of moisture absorbed by the oven-dried samples, the latter being transported in a desiccator and rapidly weighed. Four replicates were prepared for each fungus species tested and where possible several different strains made up these replicates.

A drawback to this method is that both the cellulose substrate and the resultant fungus growth are weighed together and the final result is expressed as loss in dry weight due to the loss of carbon dioxide by respiration. It is impossible by this method to determine the actual percentage loss of cellulose and also the dry weight of fungus produced. Furthermore, the filter pads retain the mineral salts which crystallise out during oven-drying and thus give a false weight reading. This disadvantage is difficult to eradicate and no attempt was made to remedy this fault, however, an attempt was made to try to express the fungal mycelial weight and percentage loss of cellulose using the following modified method.

Whatman Chromedia cellulose powder in approximately 5 gm. samples was oven-dried, weighed to constant weight amd placed in 150 ml. conical flasks. 50 ml. aliquots of a mineral salts solution were added and autoclaved as above. During incubation the cellulose powder settled down to form a thick layer on the bottom of the conical flask whilst the inoculum agar plug floated several millimetres above, on the surface of the mineral solution. In order for growth to occur cellulases must permeate

into the medium and attack the cellulose which would permit the absorption by the fungal mycelium of the breakdown products of the cellulose. Normally there was a mat of fungal mycelium floating on top of the medium, separated from the cellulose powder layer by a visible gap. The mat was lifted clear with a spatula, dried and weighed. Initial attempts to separate the two fractions by filtration proved impracticable. The flask plus cellulose was oven-dried, and the whole weighed to constant weight. However, inaccuracies were still evident in this method; contamination of the fungal mat by cellulose particles during separation and in some cases a thin, subterranean mat was formed over the surface of the cellulose layer making separation impossible. Some species showed no contact between the two layers and the results from these are presented in Table 29. In later experiments less cellulose powder was used (3 gm.) in only 20 mls. of mineral solution and this allowed intimate contact between the fungal mat and the cellulose layer, which resulted in a much denser subterranean mat but having less surface sporing growth. It was, therefore, decided to use the latter method in the majority of tests and the results could be more

directly compared with those obtained for the filter paper method.

N.B. The following mineral salts solution was used in the methods described:

$NaNO_3$, 5 gms.; K_2HpO_4, 1 gm.; $MgSO_4.7H_2O$, 0.5 gm.; $Ca(NO_3)_2.4H_2O$, 0.01 gm.; $FeCl_3$, 0.001 gm.; yeast extract, 0.5 gm.; tap water, 1 litre,(pH 6.5).

Results

The inoculum used for each determination was taken from a culture originally growing on cellulose powder agar and hence, the cellulases, when present, would be in an active state of production. The strains of species tested were the same as these employed in section i, giving some standardisation for purposes of comparison.

The species which were unable to utilise cellulose and showed no growth on filter-paper or cellulose powder are those of Group 1 species listed in section i. However, one noticeable exception occurred: Allescheria terrestris failed to grow on either filter-paper or cellulose powder in a mineral salts solution, but in section i. it was found to clear cellulose agar slightly and was classified in the Group 3 species, i.e. cellulose decomposers.

a. Filter-paper method

Those species which were able to utilise cellulose in the form of filter-paper are listed in Table 28. The ability of the fungus to utilise cellulose is expressed: a) as loss in cellulose dry weight (in mgms.) due to respiration, after subtracting the value of the control series;

b) as percentage weight loss.

The fungi were incubated at 40°C or 45°C, depending on their optimum temperatures for growth, and weighed after a three week incubation period.

From Table 28 three broad categories of cellulolytic ability can be delimited. Species producing a weight loss of under 3% (approx. 100 mgms.) can be considered to be weakly cellulolytic. Most of the species, however, fall into the 3-7% weight loss category, and these species would appear to vary from weak to fairly strong cellulose decomposers, accounting for a 100-200 mgms. loss in weight. Two mutants of Aspergillus fumigatus, in particular the orange variety which caused nearly a 30% weight loss, would appear to be very strongly cellulolytic organisms.

Strain differences in cellulolytic ability appear

Table 28

The weight loss (and standard deviation) of filter-paper due to respiration

Species or strain	Loss in mgms. and S.D.	% wt. loss
Aspergillus fumigatus	108.5 ± 10.3	3.7
A. fumigatus, olive var.	228.2 ± 13.1	7.6
A. fumigatus, orange var.	897.8 ± 9.7	28.9
Aspergillus nidulans st.1	36.9 ± 9.3	1.2
Aspergillus nidulans st.2	102.3 ± 10.4	3.3
Cephalosporium sp. 1	150.2 ± 5.6	4.6
Cephalosporium sp. 2	132.3 ± 4.9	4.1
Chrysosporium sp. 1	172.1 ± 5.8	5.7
Chrysosporium sp. 2	110.8 ± 6.1	3.6
Chaetomium thermophile var. coprophile	138.5 ± 12.3	4.6
var. dissitum	130.2 ± 7.4	4.4
var. thermophile	143.7 ± 14.2	4.7
Chaetomium sp. 1	73.5 ± 2.5	2.4
Chaetomium sp. 2	36.8 ± 2.3	1.2
Humicola insolens st.1	92.5 ± 3.4	3.1
Humicola insolens st.2	175.3 ± 8.1	5.7
Humicola sp. 1	132.6 ± 9.6	4.4
Humicola sp. 2	98.4 ± 5.4	3.2
Myriococcum albomyces st.1	64.2 ± 12.5	2.2
Myriococcum albomyces st.2	73.5 ± 3.6	2.4
Scopulariopsis sp.	58.6 ± 5.3	1.9
Sporotrichum thermophile st.1	103.3 ± 2.6	3.3
Sporotrichum thermophile st.2	106.8 ± 6.5	3.5
Sporotrichum sp.	63.3 ± 2.4	2.0
Thermoidium sulphureum st.1	39.1 ± 2.6	1.2
Thermoidium sulphureum st.2	30.6 ± 2.6	1.2
Torula thermophila	142.5 ± 3.7	4.7
Trichophaea sp.	72.1 ± 7.7	2.4

to be significant in certain species. For example, one strain of Humicola insolens caused a 5.7% weight loss, as against 3.1% for another strain of this species. An even more marked difference is apparent between the two strains of Aspergillus nidulans tested, and obviously the mutant strains of A. fumigatus would appear to be much more cellulolytic than the normal strain used in this study. The varieties of Chaetomium thermophile, however, show very similar cellulolytic abilities.

Thermoidium sulphureum produced a dense growth on the filter-papers and stained them a dark pink colour. The filter-papers were also fragile to handle and it may be thought that extensive cellulose decomposition had occurred. However, from the results, this species would appear to be weakly cellulolytic.

b. Cellulose powder method

Few fungal species showed clear separation between the mycelial and cellulose powder layers and the results of mycelial weight and the actual weight loss of cellulose powder and presented in Table 29.

From Table 29 the actual weight loss in cellulose powder is much higher than that due to loss by

Table 29

The weight loss of cellulose powder and mycelial mat weight

Species	Mycelial wt. in mgms.	Cellulose wt. loss in mgms.	% wt. loss
Chaetomium thermophile			
var. coprophile	63.2	276.7	5.5
var. dissitum	78.4	243.4	4.9
Myriococcum albomyces st.1	58.5	162.5	3.2
Chrysosporium sp.1	86.2	265.7	5.1
Sporotrichum thermophile	50.1	203.4	4.0

respiration only (see Table 28). This is as might be predicted and the obvious differences must be due to the comparative mycelial weight which is included in the final results for the other method used, i.e. method ii.a. The percentage loss is not remarkably higher due to the fact that a larger amount of cellulose was used for this method, and hence the percentage weight loss cannot be accurately compared when different initial weights of cellulose are used, viz: approximately 3 gms. of filter paper were used in the first method (a) and approximately 5 gms. were used in the latter method (b).

The results from the variation of the cellulose-

powder method on comparison with the filter-paper method, i.e. both measuring loss in weight due to carbon dioxide evolution, were found to be very similar and hence these results are not presented. It was decided, however, to use the former method primarily to test a small range of species, of verying cellulolytic ability, in order to compare the effect of temperature on cellulase production. These results are presented in Table 30.

From Table 30, it would appear that, in general, the cellulase activity shows a close correlation with the optimum temperature for growth of a species. Humicola spp. 1 and 2 can both grow slowly at 25°C but little or no cellulose weight loss was recorded, whereas at 40°C, near the optimum for growth, cellulase activity was markedly increased. Similarly, notable differences can be seen between the results for Sporotrichum thermophile and Thermoidium sulphureum at low and optimum temperatures for growth. However, Trichophaea sp. had a slightly increased cellulase activity at 25°C compared with that at 40°C, although the optimum temperature for the growth of this species lies close to the latter temperature. Thus, the cellulases of this species would appear to be more or equally as active at

lower temperatures compared with their activity at the optimum temperature for growth.

Table 30

The effect of temperature on the cellulolytic ability of several selected species (after 3 weeks)

Species	Temperature °C.	% wt. loss
Cephalosporium sp. 1	25	3.3
	40	4.4
Humicola sp. 1	25	0.2
	40	4.1
Humicola sp. 2	25	0.3
	40	3.9
Myriococcum albomyces	35	2.1
	45	2.5
Sporotrichum thermophile	25	2.1
	40	3.6
Thermoidium sulphureum	35	0.7
	45	1.3
Trichophaea sp.	25	3.0
	40	2.7

Discussion of cellulose results

Correlations can be drawn between the two methods (i. and ii.) employed for measuring cellulolytic ability. The species which utilise cellulose can be classified into several groups, comparable with certain of the groups defined in section i., viz: weak, average and strong cellulose decomposers. These groups, however, tend to overlap and a slightly wider grouping is considered necessary. These groupings of relative measures of cellulolytic ability are presented in Table 31 with a comparison of the two methods used.

The legend is as follows:

n.g. = no growth

\+ = weakly cellulolytic

++ = weak - average

+++ = average - strong

++++ = strongly cellulolytic

+++++ = very strongly cellulolytic

Brackets indicate strain differences or when a species is a borderline case between these artifical groupings.

Table 31

A comparison of the degree of cellulolytic ability of thermophilous fungi, using two different methods

Species	Cellulose clearance	Cellulose weight loss
Allescheria terrestris	++	n.g.
Aspergillus fumigatus	++++	++
A. fumigatus, olive var.	++++	++++
A. fumigatus, orange var.	+++++	+++++
Aspergillus nidulans	++	++(+)
Cephalosporium sp. 1	+++	+++
Cephalosporium sp. 2	++	++
Chrysosporium sp. 1	++	+++(+)
Chrysosporium sp. 2	++	++
Chaetomium thermophile	+++(+)	+++
Chaetomium sp. 1	+	+
Chaetomium sp. 2	++	+
Humicola insolens	++++	+++(+)
Humicola sp. 1	++	++
Humicola sp. 2	++(+)	+(+)
Myriococcum albomyces	++++	+
Scopulariopsis sp.	+	+
Sporotrichum thermophile	+++(+)	++
Sporotrichum sp.	+	+
Thermoidium sulphureum	+	+
Torula thermophila	++++	+++
Trichophaea sp.	+	+

From Table 31 a number of comparisons can be drawn between the two methods used for a wide range of species. Those species which faintly cleared cellulose powder agar also correspondingly caused only small amounts of cellulose breakdown, in the form of filter-paper, and hence these species can be classified as being weakly cellulolytic. The distinctions between weak and strong cellulose decomposers are easy to define using these two methods, but the variable amounts of cellulolytic ability which occur between these two standards (weak and strong) are more difficult to separate. The artificial classification adopted is an attempt to delimit these varying standards of cellulolytic ability, but obviously no hard and fast rules can apply. The majority of species fall between the two extreme groups of weak and strong cellulose decomposers and can be classified in a weak - average - strong complex.

From an analysis of the results from the two methods compiled in Table 31, the strongly to very strongly cellulolytic species can be separated, viz: Aspergillus fumigatus (olive and orange varieties), and to a lesser extent Humicola insolens, Torula thermophila, Chaetomium thermophile, Cephalosporium sp. 1 and Sporotrichum

thermophile. Due to the difficulty of obtaining an accurate measure of cellulolytic ability when precise clearance zones are not produced using method i.; certain species such as Aspergillus fumigatus (normal strain) and Myriococcum albomyces would appear to have a much greater cellulolytic ability by this method than by the more accurate weight loss method. However, certain other species, e.g. Chrysosporium sp. 1 show the reverse trend, i.e. weakly cellulolytic by method i. but fairly cellulolytic by method ii. Furthermore, Allescheria terrestris was found to clear cellulose agar slightly but failed to grow, and therefore cause any weight loss with filter-paper as the sole carbon source.

These results are at variance with the results obtained by previous workers. Henssen (1957) reported Sporotrichum thermophile as being weakly cellulolytic and did not record any cellulolytic ability for Aspergillus fumigatus. The author did, however, find Humicola (insolens) to be strongly cellulolytic. The high cellulolytic ability of the latter species was further endorsed by Chang (1967) who also found Aspergillus fumigatus, Chaetomium thermophile (very strong) and Sporotrichum thermophile to be strong

cellulose decomposers in the present study (see Table 31). A further point of agreement with the latter author's results was the fact that Thermoidium sulphureum was found to be weakly cellulolytic, whilst Mucor pusillus, Talaromyces duponti and Thermomyces lanuginosus were unable to use cellulose at all. The strains of Chaetomium thermophile tested during the present study do not appear to be as strongly cellulolytic as those used by Chang, who reported this species was able to break down much more cellulose than any other thermophilous fungal species that she studied, over a three week incubation period. Furthermore, the actual amounts of cellulose breakdown she listed were far higher than those recorded during the present study, except for the orange variety of A. fumigatus which caused far more cellulose breakdown than any of the other species tested, and it must be supposed that this is due to strain differences.

In order to evaluate the ability of the species tested during this study, to break down cellulose, it is necessary to compare the quantitative results of this study with those reported by other workers. Garrett (1962) found strains of Rhizoctonia solani to cause an average weight loss of about 10% when grown on filter-

paper (i.e. method ii.a), over a six week incubation period, and he considered this to be a relatively strongly cellulolytic species. Garrett (1963) compared the cellulolytic ability of five fungal species causing cereal foot rots by the filter-paper method, and recorded weight losses of 1.1-14.8%. He considered 1.1% to be low but significant and the upper limit (14.8%) to be an exceptionally high level of cellulolytic ability. The majority of the species tested during the present study fall into the range reported by Garrett for four of the species, i.e. 1-8.5% weight loss (see Table 28). In comparison with these results A. fumigatus (orange variety) can be regarded as an extremely efficient cellulose decomposer, causing nearly a 30% weight loss over a three week incubation period.

The relatively high cellulolytic ability of Chaetomium thermophile, Humicola insolens and Torula thermophila would enable these species to grow on dung, manure and compost which frequently have high cellulose contents. This is borne out by the high occurrence of these species in such habitats (see Chapter II). The occurrence of Humicola sp. 1 and 2 in manure heaps may be enhanced by their cellulolytic ability. Furthermore

the wide distribution of Aspergillus fumigatus and Chaetomium thermophile in a variety of habitats may be partly due to their ability to decompose cellulose rapidly and thus compete with other species when conditions are favourable. Further the high cellulolytic ability of the orange variety of Aspergillus fumigatus may account for the dominance of this species in the thermophilous mycoflora of mountainous heathland. The dense piles of Calluna litter would be very rich in cellulosic material and this species would be able to compete successfully with other soil fungi, especially in view of its wide temperature range for growth. The occurrence of Myriococcum albomyces and Sporotrichum thermophile in a number of habitats may, in part, be due to the presence of cellulases, so enabling them to colonise and decompose cellulose in the soil competitively, whenever environmental conditions are suitable.

The fact that cellulases are active at temperatures conducive to the thermophilic growth habit has been shown and also that they are more active at the optimum temperatures for growth than at lower temperatures. However, Trichophaea sp. was found to have a wide temperature range of cellulase activity and the rate

of activity was constant over this range. Loginova and Tashpulatov (1967) found that the cellulases of Aspergillus fumigatus were active up to 60°C and this would be an important factor in the colonisation of warm habitats by this species.

SUMMARY

An ecological study was made of the thermophilous mycoflora of a wide variety of habitats. These habitats were divided into natural areas, semi-natural areas and industrial areas. Fungi were isolated using the soil plate method of Warcup and incubation temperatures of 45-50°C were employed.

Thermophilous fungi were found to constitute a small but essential part of the microflora of normal soils, although they occurred in greater density in semi-natural areas, i.e. manures, composts, sewage and birds' nests. Particular emphasis was placed on an investigation of industrial habitats and especially on an extensive survey of the thermophilous fungi occurring in coal spoil tips. Certain of these spoil tips were found to be actively burning and high surface temperatures were frequently recorded. These so-called "warm areas" were often colonised by several moss or grass species, and proved to be particularly rich as a source of thermophilous fungi. A variety of unusual species was unearthed and it is thought that several of these species may be adapted to the conditions existing in the coal spoil tip environment. The numbers of fungal

isolates recorded from these habitats were not, however, significantly higher than those obtained from normal soils. It is thought that the organic matter content of the coal spoil tips may be the uppermost factor in determining the size of thermophilous fungal populations.

From a comparison of the fungi present in each habitat the thermophilous fungi have been classified into defined groups depending on their frequencies of isolation and occurrence. The most commonly isolated species were found to be Aspergillus fumigatus, Mucor pusillus and Thermomyces lanuginosus, comprising over 70% of the total isolations.

A study of the seasonal variation of thermophilous fungi was also undertaken. Seasonal variation in a coal spoil tip profile was shown to occur, with high numbers of isolates and species being recorded during the winter and to a certain extent during the summer months, and minima being recorded during spring and autumn. The numbers of thermophilous fungal isolates and species were found to decrease markedly with increase in depth, although a few species were found to occur more frequently in the lower horizons, and reasons are discussed.
A comparable seasonal variation was also observed in the

thermophilous fungal populations of the air spora. The species with the highest frequencies of isolation were found to be the most commonly isolated from soils. These species all possess a great capacity for producing numerous asexual spores.

A discussion of the terms thermophilic and thermotolerant is given in relation to the present context. In order to determine the degree of thermophily of the various species isolated during this study, temperature-growth relationships were examined in detail, and minima, optima and maxima were determined for all the species. A more comprehensive graphical analysis of these relationships for the rare or unusual thermophilous fungal species is presented. Species have been sub-divided into groups depending on their cardinal temperatures for growth, and several previously unreported species, viz: Chrysosporium sp., Penicillium sp. and a Rhizopus sp., were included in the true thermophiles, comprising sixteen species. Approximately thirty species were assigned to the various groupings of thermotolerant fungi, and there was a tremendous range in their cardinal temperatures for growth. Strongly thermotolerant species were found to be almost indistinguishable from weak thermophiles on the

basis of their temperature-growth relationships and it is thought that the arbitary definition of thermophily (Cooney and Emerson, 1964) in this case becomes ambiguous.

The taxonomic positions of all the isolated species is discussed and considerable emphasis is given to rare or previously undescribed species. One new species, Penicillium argillaceum, is described and several new species are proposed, viz: Calcarisporium (thermophile), Chrysosporium (thermophile), Penicillium (album), Sphaerospora (thermophile) and Talaromyces (leycetti). Several new combinations are also discussed, viz: Acrophialophora (Paecilomyces) fusisporus and Scolecobasidium (Diplorhinotrichum) gallopavum, these two species are new to the British Isles (and to Europe in general). The latter species has previously only been isolated from brain infections of turkey poults. Other species new to the British Isles are also described: Geotrichum arbustum, Mortierella wolfii and Myriococcum albomyces. A species showing taxonomic affinities with those of the genera Geniculosporium, Nodulosporium and Tritirachium is described and its classification for the moment has been set aside, although it is thought to represent a new genus. A number of other species are

also described but because of a lack of information they have not been fully classified, these include a Chaetomium species, two Humicola species, a Phialophora species, a Rhizopus species, a Thielavia species and a Basidiomycete species possessing an arthrospore-like asexual stage. It is hoped to comment further upon these species at a later date. A number of strains of Aspergillus, thought to be unusual varieties or mutants of Aspergillus fumigatus, are also described.

The cellulolytic ability of a range of thermophilous fungal species was investigated using two different methods, namely direct measurement of cellulase activity, which involved clearance of cellulose agar, and also determination of loss in weight of cellulose due to carbon dioxide evolution. An attempt was made to measure the amount of cellulose breakdown directly by separating the fungus mycelium from the cellulose substrate, but this was only partially successful. Cellulolytic ability was found to be greatest at the optimum temperature for growth of the species.

The fungi were divided into four groups on the basis of their cellulolytic ability, and an orange-coloured mutant of Aspergillus fumigatus was found to

be by far the most vigorous cellulose decomposer of all the species tested. A discussion relating cellulolytic ability with species occurrence is included.

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